Risk factors for *Staphylococcus capitis* pulsotype NRCS-A colonisation among premature neonates, Neonatal Intensive Care Unit, Dunedin Hospital

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Abstract

Background

Coagulase-negative staphylococci (CONS) are the most common cause of bloodstream infections in neonatal intensive care units (NICUs). *S. capitis*, a species of CONS, has recently emerged as a common cause of neonatal disease, in some NICUs causing up to 40% of all bloodstream infections. A clone of *S. capitis* called NRCS-A, which is multidrug resistant and capable of persistence in NICUs, has been isolated from neonates worldwide. Most studies of *S. capitis* NRCS-A have focused on the genome structure, or risk factors for bloodstream infection. We conducted both a retrospective and prospective study among neonates admitted to the Dunedin Hospital NICU to investigate the risk factors of *S. capitis* skin colonisation and transmission.

Methods

Our studies used the results from an ongoing Dunedin Hospital NICU *S. capitis* surveillance system. We collected data on baseline and weekly neonatal exposures, along with *S. capitis* surveillence test results. We collected weekly data on exposures such as medication use, medical history, procedures and devices, enteral feeds, weight, type of beds, and bed spaces. Additionally the prospective study collected weekly data on family and staff contact.

The retrospective study analysis categorised participating neonates each week as a case or a control depending on whether the neonate became colonised with *S. capitis* or remained non-colonised, respectively. We matched control data to case data based on calendar time. A nested case-control analysis with conditional logistic regression was used to investigate the differences between cases and controls for baseline and weekly exposures. A full analysis of the ongoing prospective study will be performed when data collection is complete.

Results

A progress report of the prospective study described the baseline characteristics of the 13 participants: 1 case and 12 controls. Our retrospective study nested-case control analysis included 26 cases and 203 controls.

Factors associated with an increased risk of *S. capitis* colonisation included oral sodium chloride use (OR 6.1, 95% CI 1.4-27.1, p=0.02), diagnosed patent ductus arteriosus (OR 2.9, 95% CI 1.1-7.9, p=0.04), diagnosed chronic lung disease (OR 4.8, 95% CI 1.1-22.3, p=0.04), and requirement of invasive mechanical ventilation (OR 3.4, 95% CI 1.1-10.4, p=0.03).

Factors associated with decreased risk of *S. capitis* colonisation included being born as a part of a multiple birth (OR 0.14, 95% CI 0.04-0.40, p=<0.001), having an area of inflamed skin (OR 0.31, 95% CI 0.13-0.70, p=0.005), having an inflamed axilla (OR 0.31, 95% CI 0.13-0.70, p=0.005), and enteral feeds with formula (OR 0.29, 95% CI 0.08-0.99, p=0.05).

Conclusions

We found that co-morbidities, such as patent ductus arteriosus and chronic lung disease, or devices, such as invasive mechanical ventilation, were associated with an increased risk of *S. capitis* colonisation. Having an inflamed axilla, and antimicrobial use were independently more common among neonates that ever became colonised than neonates that never became colonised, although in the nested case-control analysis neither were associated with a statistically significant increased risk of *S. capitis* colonisation. Overall, our retrospective study showed that neonates with comorbidities, which may require more management by medical staff and equipment, were associated with an increased risk of *S. capitis* colonisation.

Preface

The Dunedin Hospital NICU initiated a *S. capitis* surveillance system in September 2013, although no epidemiologic study had been performed to identify risk factors for colonisation. Louise Thorn, Master of Public Health candidate (MPH) designed both a retrospective and a prospective study to investigate the risk factors for *S. capitis* colonisation among neonates in the Dunedin Hospital NICU.

The roles of the candidate:

- Established the objectives of both the retrospective and prospective studies based on communication with Dunedin Hospital NICU staff and supervisors.
- Prepared and obtained Department of Preventive and Social Medicine, University of Otago, Research Advisory Committee (RAC) approval.
- Prepared and obtained Health and Disability Ethics Committee (HDEC) approval for the prospective study. Additionally, sought and received approval for minor amendments to the original ethics approval.
- Designed and edited the case report forms (CRFs) for both the retrospective and prospective studies.
- Responsible for ensuring the Dunedin Hospital NICU staff were coded appropriately to protect anonymity in the prospective study.
- Designed and created a REDCap (service version 8.2.0, Vanderbilt University, Tennessee, USA) online database for data entry for both the prospective and retrospective studies.
- Performed the prospective study data entry from the CRFs to the REDCap database.
- Coordinated the prospective study, including regular meetings with data collectors for quality control.
- Undertook all retrospective study data collection from medical records, and data entry into the REDCap database.
- Did basic statistical description of the prospective study participants as a progress report of the ongoing prospective study.

- Matched controls to cases in the retrospective study nested case-control analysis.
- Performed all statistical analyses of the retrospective study data with guidance from a statistician.
- Full write up a Masters of Public Health thesis.
- Will prepare and submit a paper for publication in peer-reviewed literature describing the retrospective study.

The roles of others involved in the studies:

Professor John Crump (supervisor) contributed to study design, preparation of the proposal for RAC approval, sourced questionnaires from other *Staphylococcus* transmission and disease studies, and advised on design and analysis of both studies and content of the CRFs. Prof Crump provided advice and guidance throughout the course of both studies. Prof Crump also contributed considerably to reviews and comments on drafts of the thesis.

Associate Professor Roland Broadbent (supervisor) contributed to establishing relationships between the candidate and the NICU staff, advised on both the objectives of the study and content of the CRFs. Assoc. Prof Broadbent provided codes and timetables for the registrars and consultants. For the retrospective study, he ordered the medical records from Dunedin Hospital storage, provided advice regarding the specific sources of data for each section of the CRFs, and sourced access to the hospitals online database for data that were not available among medical records. He also reviewed drafts of the thesis.

Dr James Ussher (supervisor) contributed to the preparation of the prospective study HDEC approval, advised on the study objectives, and the content of the CRFs. He also provided the document of neonates *S. capitis* surveillance test results during the period of the retrospective study, and provided data on the number of *S. capitis* invasive infections during both study periods. Dr Ussher reviewed drafts of the thesis and was particularly helpful with advice for the laboratory methods and microbiology sections.

Assoc. Prof Katrina Sharples (supervisor) contributed to study design, advised on data collection, and provided the sample size calculations. Assoc. Prof Sharples also provided advice on the statistical methods for both the prospective and retrospective analyses. Assoc. Prof Sharples also reviewed drafts of the thesis.

Dunedin Hospital NICU charge nurse Juliet Manning was involved in the prospective study, which included: advice on the content of the CRFs, helped pilot the CRFs, provided nurse codes, trained three other nurses in data collection, and had primary responsibility for data collection. Juliet Manning also provided the numbers of ineligible neonates admitted to the Dunedin Hospital NICU and the reasons for ineligibility, and data on the total admissions for the prospective and retrospective study periods.

Acknowledgements

I would like to acknowledge my supervisors Prof John Crump, Assoc. Prof Roland Broadbent, Assoc. Prof Katrina Sharples, and Dr James Ussher for their endless expertise in diverse fields. They helped me in many ways including study design, establishing relationships with NICU staff, completing ethics approvals, ordering medical records, access to *S. capitis* test results, and answering countless statistical questions. Additionally, the edits, advice, and grammar checks on the numerous drafts of my thesis were indispensable and I thoroughly appreciate it.

I would also like to thank the Dunedin Hospital NICU staff, especially Juliet Manning who helped with the establishment and continuation of the prospective study. Additionally, thank you to the Dunedin Hospital clinical records team who rapidly collected the medical records for the retrospective study.

I would like to thank the staff in the Centre for International Health and Injury Prevention Research Unit, and the Women's and Children's Health department for making me feel welcome throughout the time I spent in both of the offices. I would also like to thank the Department of Preventive and Social Medicine for their support.

Prevention and Response Branch, Division of Healthcare Quality Promotion, US Centers for Disease Control and Prevention provided me with questionnaires from previous studies of *Staphylococcus* transmission and disease which were an immense help for development of my case report forms.

Thank you to my family and friends for their endless support, especially my parents, Hannah Hampton, and Amy Richardson for their writing advice and proofreading skills.

Lastly, I would like to thank my partner Evan for his constant encouragement and belief in me. Finishing this process would have been a lot more difficult without it.

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Abbreviations

US CDC	United States Centres for Disease Control and Prevention
CFU	Colony Forming Units
CI	Confidence Interval
CIH	Centre for International Health
CLD	Chronic Lung Disease
CONS	Coagulase Negative Staphylococci
COPS	Coagulase Positive Staphylococci
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribose Nucleic Acid
EONS	Early Onset Neonatal Sepsis
FIRS	Foetal Inflammatory Response Syndrome
GA	Gestational Age
GIT	Gastrointestinal Tract

ICU	Intensive Care Unit
ID	Identification
HIV	Human Immunodeficiency Virus
HUSS	Head Ultrasound Scan
IE	Infective Endocarditis
IUGR	Intrauterine Growth Restriction
IV	Intravenous
LONS	Late Onset Neonatal Sepsis
MALDI-ToF-MS	Matrix Associated Laser Desorption Ionisation Time of Flight Mass Spectrometry
mg	Milligram
MIC	Minimum Inhibitory Concentration
NEC	Necrotising enterocolitis
NGT	Nasogastric Tube
NHI	National Health Index
NICU	Neonatal Intensive Care Unit

NRCS-A	National Reference Centre for Staphylococci
OGT	Orogastric Tube
OR	Odds Ratio
PIA	Polysaccharide Intercellular Adhesin
PICC	Peripherally Inserted Central Catheter
PDA	Patent Ductus Arteriosus
PPE	Personal Protective Equipment
PROM	Premature Rupture of Membranes
RDS	Respiratory Distress Syndrome
ROP	Retinopathy of Prematurity
SCC*mec*	*Staphylococcus*-specific gene Cassette
SCL	Southern Community Laboratories
sd	standard deviation
TEWL	Transepidermal Water Loss
TPN	Total Parental Nutrition
UAC	Umbilical Artery Catheter

UK	United Kingdom
UI	Uncertainty Interval
USA	United States of America
UTI	Urinary Tract Infection
UVC	Umbilical Venous Catheter
WGS	Whole Genome Sequencing
WHO	World Health Organisation
VLBW	Very Low Birthweight

1 Introduction

Although common contaminants of blood cultures, CONS are the most common cause of bloodstream infections and sepsis among neonates in NICUs (1-5). CONS are commensals of the skin and mucous membranes of humans and animals, but may act as opportunistic pathogens when they enter the bloodstream or other normally sterile sites (6). *S. epidermidis* is the species most often implicated in CONS disease (7). However, *S. capitis* is emerging as an important cause of bloodstream infections in neonates (8).

A strain of *S. capitis*, called *S. capitis* pulsotype NRCS-A, was identified not only colonising premature neonates but also causing serious infections in a hospital NICU in France in 2012 (9). Subsequently, *S. capitis* NRCS-A was identified to be widespread not only in NICUs elsewhere in France but also worldwide (9, 10). The homology between the strains isolated from geographically diverse NICUs suggested global dissemination of the *S. capitis* pulsotype NRCS-A clone. Studies have investigated the genome structure of *S. capitis* pulsotype NRCS-A, to understand the clones ability to survive and persist in the NICU environment (10, 11). Notably, the NICU-specific *S. capitis* clone is resistant to methicillin, aminoglycosides, and has decreased susceptibility to vancomycin (11). *S. capitis* NRCS-A causes up to 40% of all bloodstream infections in NICUs where it is endemic (9, 12).

Studies have shown that intravascular catheter use is a risk factor for neonatal bloodstream infections, while there is also some evidence for bacterial translocation to the blood through the gastrointestinal tract (12-14). Colonisation is likely a prerequisite for infection with *S. capitis* pulsotype NRCS-A, so studies of transmission focused on colonisation are needed to inform prevention and control. Previous studies have identified low birthweight, vancomycin treatment, and almond oil as a skin emollient as risk factors for *S. capitis* colonisation (15, 16).

Although Butin et al. identified low birthweight, and vancomycin use as independent risk factors for gastrointestinal tract (GIT) colonisation (16), they did not collect data on exposures related to transmission of *S. capitis*. Therefore, regardless of modification

of antimicrobial use in the NICU, the neonates may still be exposed to the source of *S. capitis* and those born at low birthweight will remain at risk of colonisation. Gras-Le Guen et al. identified almond oil as a risk factor for colonisation of blood, aspirate, and abscesses of neonates (15). However, they did not establish temporality so could not identify whether the almond oil was contaminated before or after the neonates were colonised. Moreover, *S. capitis* continued to be isolated from the Nantes hospital NICU, therefore almond oil was not the only source, or reservoir of *S. capitis*. Although the Gras-Le Guen study suggested that staff members were the source of transmission from the almond oil to the neonates, no studies to our knowledge have yet investigated staff members as a source of *S. capitis* transmission.

S. capitis pulsotype NRCS-A has been isolated from neonates in the Dunedin Hospital NICU since 2007 (personal communication, James Ussher and Roland Broadbent). We sought to identify the risk factors for *S. capitis* colonisation among neonates in the Dunedin Hospital NICU using both retrospective and prospective studies. To our knowledge, our studies were the first known epidemiologic studies to examine the risk factors of *S. capitis* skin colonisation and the source of transmission. The studies aimed to identify the source of colonisation among people and the NICU environment. Additionally, the ongoing prospective study traces staff, bed space, incubator, and stethoscope contact with neonates. Identification of the source of transmission may provide evidence for modification of NICU practices to reduce transmission of *S. capitis* pulsotype NRCS-A and in turn the proportion of neonates who develop *S. capitis* bloodstream infections.

2 Literature review

2.1 Coagulase-negative staphylococci, *Staphylococcus capitis*, and *Staphylococcus capitis* pulsotype NRCS-A

2.1.1 Ecology in humans, animals, and the environment

CONS make up part of the normal skin and mucosal flora of humans and animals (6). *Staphylococcus* species prefer to colonise moist areas of the human body such as the axillae, nares, and gastrointestinal tract (17, 18). The skin is a heterogeneous organ with variation in skin thickness, and an abundance of glands and hair follicles. This variation encourages niche differentiation of bacterial species (6) and some CONS species have evolved to colonise particular skin areas. For example, among adults *S. auricularis* predominantly colonises the auditory canal (19) and *S. saprophyticus* the perineal region (20). By contrast, *S. epidermidis* colonises the entire skin surface (21, 22).

In addition to being an important part of the human skin flora, CONS colonise the skin and mucous membranes of non-human animals including cattle, cats, dogs, goats, horses, pigs, poultry, and sheep (6, 23, 24). Certain CONS may be adapted or restricted to specific animal species (25), while other CONS are capable of colonising both humans and a range of animal species (23). For example, *S. kloosii* is a CONS species predominantly found on animals, including farm animals, wild animals, and some marine animals (21, 26, 27). *S. kloosii* has rarely been isolated from humans (27). Transmission of CONS can occur from animals to humans, sometimes posing a public health threat (28). For example, *S. haemolyticus* colonises cats, dogs, horses, and human skin (21, 29). In the hospital setting *S. haemolyticus* is an important cause of severe healthcare-associated infections (21).

CONS have been isolated from the environment as well as their living hosts. In the farming industry, CONS colonise niches such as milking liners, floors, and sawdust (30, 31). CONS have also been isolated from food such as dry sausages, raw milk, and cheese (32). CONS in animal source foods may originate from the food animals

themselves. Others, such as *S. carnosus*, may be added during food production to produce flavor in food such as sausages and cheese (32-34). In the hospital environment, CONS can be isolated from the air and surfaces (35). One study by Neely et al. showed that CONS can survive on cotton, polyester, and polyethylene surfaces in hospitals (36). Neely et al. demonstrated that CONS persisted on polyethylene surfaces for over 90 days (36). While studies demonstrate that CONS are capable of survival and persistence in the hospital environment, none demonstrate growth and replication independent of humans. This suggests that humans and animals rather than the environment are the reservoirs for CONS, but that the environment may serve as a transmission source.

In 1975, Kloos and Bannerman classified several new species of CONS (22). One of these species, *S. capitis,* was named after the human scalp from where it was first isolated (22). The 1975 study included 19 adults aged from 14 through 61 years, and 21 children aged from 2 through 12 years (22). From this sample of adults and children, *S. capitis* was isolated from 26 (65%) of 40 individuals but the proportion from children was not provided (22). Among the 26 individuals that carried *S. capitis*, 47 isolates were recovered (22). The strains of *S. capitis* were predominately isolated from the head, with a small number from the nares, axillae, arms, and legs (22). In studies since 1975, *S. capitis* has rarely been isolated from adults, and more commonly found on infants and children (9). Bannerman et al. identified a *S. capitis* subspecies named *S. capitis* subsp. *urealyticus*, named for its positive urease activity (37). The *S. capitis* described by Kloos et al, in 1975 was designated *S. capitis* subsp. *capitis* (37). Bannerman et al. found that while both *S. capitis* subsp. *urealyticus* and *S. capitis* subsp. *capitis* were most frequently isolated from the head, *S. capitis* subsp. *urealyticus* was also isolated from other unspecified areas of the body (37).

As with other species of CONS, *S. capitis* colonises animals as well as humans. *S. capitis* has been isolated from domestic animals such as cats, dogs, and horses (6), and from goats (23). *S. capitis* has been isolated from the hospital environment, particularly in neonatal intensive care units (NIUCs) (38). In a study by Gras-Le Guen et al, *S. capitis* was isolated from a refillable bottle of almond oil used as a skin emollient (38). However, while the Gras-Le Guen study showed survival of *S. capitis* in the almond oil

it did not evaluate multiplication, so the role of almond oil as a possible *S. capitis* reservoir is unknown.

Ecology in neonates

CONS are the most common commensal isolated from skin and mucosa of neonates (39). Within the first days or weeks after birth, premature neonates are rapidly colonised with CONS (40). In a 1992 study of 10 neonates, Savey et al found that at four to five days after birth CONS were isolated from each of 18 neonatal skin sites sampled, and on average made up 81% of the neonates total skin flora (41). CONS are also the earliest and most abundant colonisers of the GIT (42, 43). The species of CONS depends on skin and environmental contacts (8, 41). For premature infants in NICUs, colonisation is often with bacteria endemic to the unit or those that have specific factors that facilitate colonisation success (40). Although studies have isolated CONS species from neonates skin, the pattern of niche differentiation of specific species was not clear (41, 44, 45). Therefore, currently niche differentiation of CONS among neonates cannot be determined. I identified six studies that sought to determine which body areas of neonates were colonised with the most abundant CONS populations (Table 1). Studies focused entirely on healthy infants rather than premature neonates were excluded. Evidence suggests substantial differences in types of CONS species that colonise healthy and premature infants (41).

Table 1: Studies of coagulase-negative *Staphylococcus* skin colonisation among neonates, worldwide, 1989-1996

Author(s), year	Participants	Study design	Skin sites tested	Sites populated with the highest CFU/area
D'Angio, 1989	18	Longitudinal	Axilla, ear, nasopharynx, and rectum.	Axilla, and ear.
Keyworth, 1992	9	Longitudinal	Forehead, chest, periumbilical region, back, upper and lower leg, and upper and lower arm.	No variation in numbers across sites.
Savey, 1992	10	Cross-sectional	Skin: scalp, armpits, neck fold, umbilicus, inguinal folds, anal cleft, lumbar area, palms of hands, inside elbows, soles of feet, and popliteal spaces. Mucosa: external auditory meatus, nasal cavities, external genital organs, pharynx, rectum, and faeces.	Skin: umbilicus, neck folds and inguinal folds. Closely followed by elbows, and axilla. Mucosa: faeces, anus, and external genital organs.
Bialkowska-Hobrzanska, 1993	6	Cross-sectional	Umbilicus, anterior nares, foot, scalp, and rectum.	Umbilicus, anterior nares, and foot.
Bertone, 1994	50	Cross-sectional	Skin covering the jugular, subclavian, umbilicus, and femur.	Jugular
Eastick, 1996.	10	Longitudinal	Ear (anterior and posterior), anterior nares, axilla, forearm, lower leg, and faeces.	Faeces, posterior ear, and axilla.

The results from the six colonisation studies were inconsistent. Two studies found that CONS were the most abundant at the umbilicus (41, 46), while the remaining studies showed that CONS were more abundant at the axilla, ear, or the skin covering the jugular (47, 48). The discordance is likely due to small sample sizes, little crossover of the areas swabbed, and different methods and a lack of standardisation for the surface areas swabbed. The majority of studies did not include a follow-up, so changes in colonisation over time could not be assessed despite fluctuation in microflora being widely recognised (44). Day-to-day variation of bacteria isolated from neonates may be due to the repetitive inoculation of bacteria from staff and the environment through frequent contact (49). The differences between studies of CONS colonisation of

neonates may also be due to different swabbing techniques, different infection control procedures, or different ages of participants.

Of interest, all studies that collected faecal samples found larger CFUs per mg of CONS in the faeces compared to other skin or mucosal sites (41, 44). However, the variation in CFUs/area among skin sites suggests that there is no predominant area of CONS colonisation and that further more standardised research is needed. Alternatively, it is possible that niche differentiation is not established until later in life. Spread of CONS from one site to another on the same individual is especially likely among neonates due to regular handling by staff (49). Additionally, premature neonates have predominantly dry skin that has not differentiated between sites whereas adult skin includes diverse characteristics ranging from oily to dry (50). It is likely that CONS species are found on all areas of the body then specialise to niche areas later in infant development. However, a longitudinal study with a large sample size, swabbing many different sites on the body needs to be done to further understand CONS niche colonisation on neonates.

2.1.2 Microbiology

In 1940, R. W. Fairbrother categorised the *Staphylococcus* genus into two groups: CONS and coagulase-positive staphylococci (COPS) (51). The classification was made based on whether or not the *Staphylococcus* produced the coagulase enzyme, detected by coagulation of blood. Fairbrother designated all CONS strains to the species '*S. saprophyticus'* and all COPS to '*S. pyogenes*' (51). The idea behind the classification was to distinguish between 'non-pathogenic' CONS, and COPS, which at the time were the only species thought to be capable of clinical disease in humans (51). From the 1970s onwards, phenotypic and genotypic identification methods improved. These advances resulted in the reclassification of staphylococci, including the designation of more species. By 2014, more than 40 species of staphylococci had been identified. Most were classified as CONS, with the COPS group including seven species (52).

Following the 1970s, studies investigated the clinical aspects of the staphylococcal species. The CONS and COPS groups were found to be less clinically distinct than

previously thought. For example, *S. lugdunensis* exhibited clinical characteristics of both groups and was designated as an intermediate species (53). *S. schleiferi* included two subspecies, *schleiferi* and *coagulans*, classified as CONS and COPS, respectively (54). In the 1990s, Kloos and Bannerman showed that some CONS were clinically important. Rather than representing a group of exclusively commensal species, the CONS group exhibited both non-pathogenic species and species capable of causing serious infections in humans (21). While CONS lack common *S. aureus* toxins that cause damage to the host, CONS do have virulence factors for colonisation and persistence (11).

S. capitis is a gram-positive coccus of approximately 0.8-1.2 μm in diameter (55). They are non-motile and do not form spores (55). On trypticase soy agar with 5% sheep blood *S. capitis* has a typical appearance of a CONS with smooth, entire, glistening, and opaque colonies (56). *S. capitis* are facultative anaerobes, with an optimum growth temperature of 30-40 ºC (55). A study by Cui et al. (2013) in a hospital NICU found that *S. capitis* subsp. *urealyticus* was predominant among neonates in the NICU, could form biofilms, and was resistant to penicillin, erythromycin, and oxacillin (57). *S. capitis* subsp. *capitis* was rarely isolated from the NICU, could not form biofilms and was susceptible to penicillin, erythromycin, and oxacillin (57). The study by Cui et al, was performed among neonates in a NICU environment so may not represent all *S. capitis* strains. However, Kloos et al (1994) also showed that under constant antimicrobial pressure *S. capitis* subsp. *urealyticus* developed resistance to penicillin and erythromycin faster than *S. capitis* subsp. *capitis* (21).

Biofilm formation is one of the main persistence factors of CONS (1, 2). Biofilms are a conglomeration of bacterial cells protected by an extracellular polysaccharide matrix that adhere to surfaces (7). Bacteria produce molecules that enable them to adhere to either biotic or abiotic surfaces such as human skin or intravascular devices, respectively (58, 59). After adhesion, other molecules cause the accumulation of cells and the maturation of the biofilm. Polysaccharide intercellular adhesin (PIA) is the main molecule that facilitates aggregation of bacterial cells (60, 61). PIA is considered a major functional component of staphylococcal biofilms (60, 61). Maturation of the biofilm results in a non-selective barrier that protects the bacteria from both the host

immune system and from antimicrobials (62-64). The final step of the biofilm is the detachment and dispersal of bacterial cells (65). If the biofilm has formed on an intravascular device, the bacteria can slough off into the bloodstream (65). The genes that encode biofilm formation are contained in operons such as the *ica* operon (*icaADBC*), and include the gene for PIA (35). *S. epidermidis* is the most common CONS species implicated in biofilm-associated infections (66). The *icaADBC* operon is commonly found in *S. epidermidis* isolated from patients with bloodstream or urinary tract infections (UTIs) in the hospital, but rarely found in isolates outside of the hospital (66) which suggests that the *icaADBC* operon may have a role in survival in the hospital though might not be as necessary in other environments.

Antimicrobial resistance mechanisms allow microbes to evade the bacteriocidal or bacteriostatic effects of antimicrobials (67). Resistance mechanisms include biofilm formation, cell wall thickening, decreased entry into the cell through porin loss, increased removal from the cell via efflux pumps, modification of the antimicrobial target, and the production of enzymes that inactivate antimicrobials (67-69). Bacteria can become resistant through genetic mutation or via the acquisition of resistance genes from other bacteria (67). Bacterial resistance to antimicrobials has increased in recent years. However, not all CONS species are resistant to the same antimicrobials (35). Different strains of the same species (11) can be resistant to different antimicrobials (35). Resistance to the beta-lactam family of antimicrobials, such as penicillin and methicillin, is now common in CONS (70, 71). Penicillin resistance in some staphylococci is encoded by the *blaZ* gene, which produces a beta-lactamase (72, 73). The beta-lactamase hydrolyses the beta-lactam ring, inactivating penicillin (73). Methicillin, a beta-lactamase-resistant penicillin, was first used in 1961 (74). Less than a year after the introduction of methicillin, methicillin-resistance was reported (74). A study in 2000 showed that 60-70% of CONS isolates were resistant to methicillin (75). Methicillin resistance is most commonly due to the presence of a modified penicillin binding protein (PBP2a) to which methicillin cannot bind (62). This modified penicillin binding protein is encoded on the gene *mecA,* which is found on a staphylococcal-specific gene cassette (SCC*mec*) (62, 76). SCC*mec* often contains other genes that confer resistance to other antimicrobials (72). SCC*mec* has been found in 11 CONS species from both human and animal sources (76).

In 2012 at the University Hospital in Lyon, France, Rasigade et al. reported a high prevalence of methicillin-resistant *S. capitis* causing late onset neonatal sepsis (LONS) in neonates in the hospital NICU (9). Early-onset neonatal sepsis (EONS) occurs at or within 72 hours after birth and LONS occurs after 72 hours (77). The classification helps with diagnosis and management because aetiology varies in predictable patterns depending on the timing of onset after birth. Rasigade et al. conducted a retrospective laboratory based survey, investigating the bloodstream isolates from neonates in the NICU and adults in the intensive care unit (ICU) from 1 January 2004 through 31 December 2009 (9). Rasigade et al. found that *S. capitis* was the most common organism isolated from the blood cultures of neonates in the NICU, but was rarely isolated from the blood cultures of adult ICU patients (9). Using pulsed gel field electrophoresis (PFGE), isolates from six other cities in France were identified as the same *S. capitis* pulsotype as the Lyon *S. capitis* isolate (9). The *S. capitis* isolates from adult ICUs were genetically diverse (9). The NICU-specific *S. capitis* pulsotype was named NRCS-A after the National Reference Centre for Staphylococci in the University Hospital in Lyon (9).

In 2016, Simoes *et al* performed the first closed genome sequence of *S. capitis* NRCS-A isolated from their NICU in Lyon, France, and compared it to *S. capitis* NRCS-A isolates from the United Kingdom (UK), Belgium, and Australia (11). Simoes et al. also compared the genome to a strain of *S. epidermidis* and strains of non-NRCS-A *S. capitis*. The French *S. capitis* NRCS-A isolate had a chromosome length of 2,522,871 bp (11). All *S. capitis* NRCS-A isolates had low G+C content (33.02% to 32.84%), and mobile genetic elements called phages and plasmids (11). All isolates of *S. capitis* NRCS-A had one phage, and the UK isolate had two other phages both of which were not intact (11). All isolates but the UK isolate had one plasmid, but the plasmids were distinct across the three isolates (11). The NRCS-A genome also included known virulence factors called Clp proteases and phenol soluble modulin (PSM) beta type 1b, both of which cleave proteins (11). *S. capitis* NRCS-A did not produce toxins or contain the secretion systems or serine proteases that are found in the *S. aureus* genome (11). Simoes et al. discovered that *S. capitis* NRCS-A contained the biofilm operons *ica*ABDC and *cap*ABC (11). While some studies have previously reported *S. capitis* to be a poor producer of biofilm compared to *S. epidermidis* (4, 72), others have shown

that *S. capitis* NRCS-A formed more biofilm than *S. capitis* non-NRCS-A strains (personal communication, James Ussher).

Simoes *et al* reported that all *S. capitis* NRCS-A isolates contained the *Staphylococcus*-specific SCC*mec* gene cassette that was completely conserved with 100% amino acid homology (11). The cassette encoded methicillin resistance via the *mecA* gene, and a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) element specific to NRCS-A, but no other resistance genes (11). Interestingly, the *S. capitis* NRCS-A cassette also contained a gene that resembled the *nsr* gene encoding nisin resistance (11). Nisin is an antimicrobial peptide secreted by some gram-positive bacterial species that reside in the gut, such as some strains of *Lactococcus lactis* (11, 78, 79). The nisin resistance gene was not found in the *S. capitis* non-NRCS-A genome (11). Using a phenotypic assay, the researchers found that *S. capitis* NRCS-A was functionally resistant to nisin (11). Resistance to nisin may confer *S. capitis* NRCS-A a competitive advantage for colonisation. All *S. capitis* NRCS-A isolates were resistant to aminoglycosides encoded by the *aacA-aphD* gene and all but the UK isolate encoded the *bla* operon and hence produced the *blaZ*-encoded beta-lactamase (11). Only the UK isolate was resistant to fusidic acid (*far1*), while the Australian isolate contained the *msrA* and *tetK* genes for erythromycin and tetracycline resistance (11).

Another study showed, when exposed to vancomycin *in vitro*, *S. capitis* NRCS-A could develop stable resistance possibly representing a vancomycin-heteroresistant phenotype (80). *S. capitis* NRCS-A developed resistance faster than other *S. capitis* strains, and resistance persisted after vancomycin selective pressure was removed (80). Resistance to vancomycin in *S. capitis* NRCS-A was associated with cell-wall thickening and subsequent resistance to other antimicrobial agents such as daptomycin and teicoplanin (10). Resistance to both methicillin and vancomycin in *S. capitis* is of concern as it leaves few choices for management of *S. capitis* infections (10). Additionally, a recent study by Butin et al. has shown that treatment of neonates with vancomycin was a risk factor for LONS with vancomycin resistant *S. capitis* (16). The conservation of genes such as the SCC-*mec* gene cassette between *S. capitis* NRCS-A from many countries suggests global dissemination of the same strain. Differences in resistance genes among

S. capitis NRCS-A strains is likely driven by varying patterns of antimicrobial use between hospitals and countries.

2.1.3 Chain of infection

The chain of infection is the path by which an agent reaches its susceptible host and causes disease. As CONS survive and multiply on the skin and mucous membranes of humans, CONS colonisation generally precedes infection. However, there are exceptions such as insertion of CONS-contaminated prosthetics. Nevertheless, understanding 'the chain of colonisation' in many cases is as important as understanding 'the chain of infection.' As there are over 30 different CONS, the chain of colonisation for CONS species are possibly diverse. Therefore, this section focuses on *S. capitis* colonisation in NICUs.

The hypothesised chain of infection for *S. capitis* pulsotype NRCS-A in hospital NICUs, shown in Figure 1, is an attempt to summarise the reviewed literature as described in the following paragraphs. A chain of infection starts with a reservoir where the agent usually lives, grows and replicates. Removal of the reservoir will result in elimination of the pathogen in a timeframe determined by persistence of the organism outside the reservoir. No study has yet confirmed the reservoir or source of transmission of *S. capitis* NRCS-A in NICUs. However, based on our wider understand of the ecology of CONS, the reservoir is likely to be human, and likely to be neonates as a previous study has shown *S. capitis* NRCS-A was rarely isolated from adults in other units in the hospital (9). In 2014 in the Dunedin Hospital NICU, the Infection Prevention and Control team swabbed staff member's external ear and thumb to assess whether any staff could be a reservoir (personal communication, J. Ussher). *S. capitis* NRCS-A was isolated from one staff member out of an unknown number of staff members tested (personal communication, J. Ussher). Since the ability of *S. capitis* to grow and multiply on healthcare workers was not tested, their role as a reservoir cannot be definitively excluded. This suggests that some staff members are possible reservoirs and sources of *S. capitis* NRCS-A. Additionally, the environment is a source but is unlikely to be an important reservoir of *S. capitis* NRCS-A. Although we have a strong

hypothesis of neonates as the reservoir, the reservoir of *S. capitis* in NICUs has not been comprehensively identified anywhere in the world.

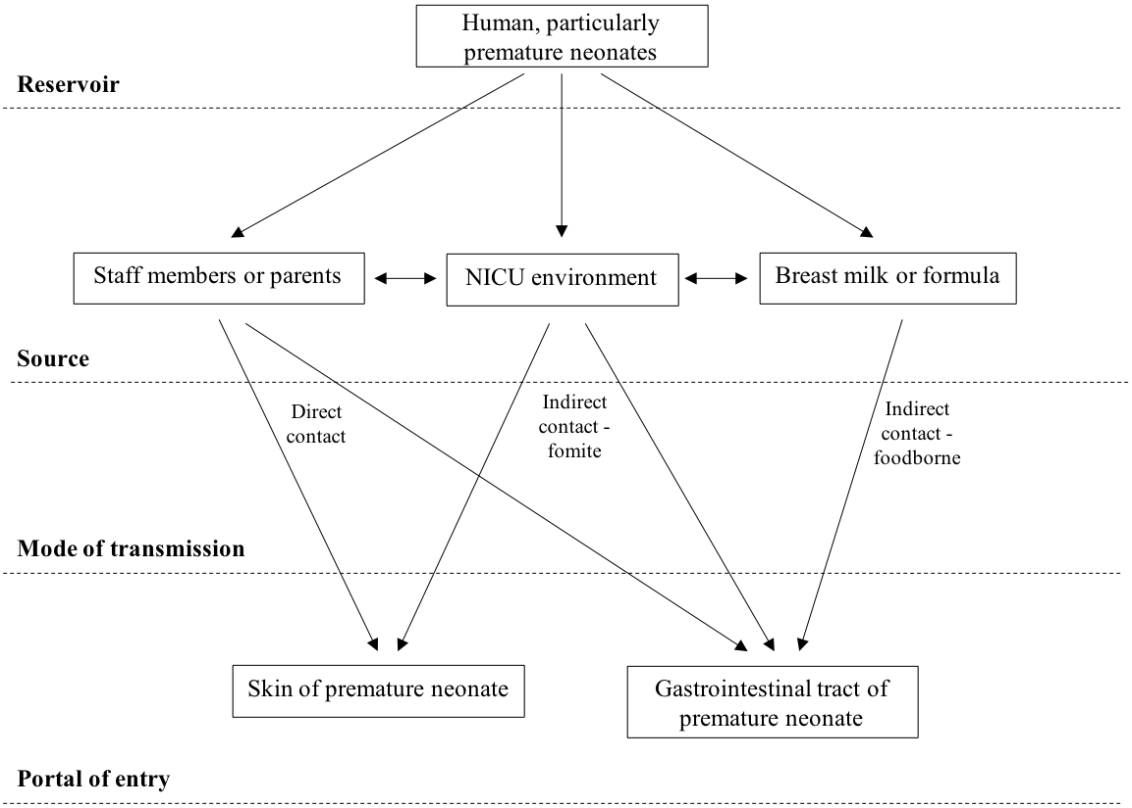

Figure 1 Hypothesised chain of infection for *S. capitis* NRCS-A in premature neonates in NICUs.

NICU – neonatal intensive care unit

The portals of exit for *S. capitis* NRCS-A are likely to be the skin, mucous membranes, or faeces. The mode of transmission, as shown in Figure 1, is either direct contact, indirect contact with fomites, or foodborne. Direct contact occurs when a human source transmits *S. capitis* to the neonate through physical contact (81). Bjorkqvist et al found no evidence that delivery mode by caesarian section versus vaginal delivery, or premature rupture of membranes (PROM) influenced colonisation with *S. capitis*, suggesting that transmission during birth is unlikely (40). However, due to the regular contact with their child, including skin-to-skin cuddles, and contact with the NICU environment when helping with cares, it is possible that parents are a source of *S. capitis* NRCS-A in the NICU.

Indirect transmission of *S. capitis* may occur via food and fomites. A fomite is an abiotic object that transfers microbial agents to the host (81). The clonality of *S. capitis* NRCS-A (section 2.1.2) may suggest a global point source outbreak, however none have been identified. A study by de Goffau et al. investigated microbial growth in neonatal incubators by comparing the microbial growth at both the cold and warm spots of incubators (82). de Goffau et al. found that the cold spots (up to 6 ºC colder than 37 ºC) of incubators had higher numbers of colony forming units (CFUs) than the warm spots (up to 2 ºC warmer than 37 ºC) (82). Additionally, the CFUs were higher from cold spots within incubators with high ambient temperature (≥34ºC) and high humidity (≥60%) compared to low ambient temperature (<34ºC) and low humidity (<60%) (82). Of the microbes isolated by de Goffau et al, staphylococci were the most common group (82). A study by Madar et al. at a hospital in Martin, Slovakia, swabbed the stethoscopes of physicians, medical students, and shared stethoscopes from ward consulting rooms (83). Madar et al, found that of the 110 stethoscopes swabbed, 101 (91.8%) were colonised with microbes, and 79 (71.8%) were colonised with CONS (83). Unfortunately, neither of these studies investigated the transmission of CONS between incubators or stethoscopes and neonates. However, if incubators and stethoscopes serve as fomites in *S. capitis* transmission, it is likely that they would facilitate the transfer of *S. capitis* to the skin rather than the gastrointestinal tract (GIT). As discussed in a previous section (section 2.1.1), Gras-Le Guen et al. identified refillable almond oil bottles as the source of *S. capitis* NRCS-A transmission in their Nantes Hospital NICU (15). However, they did not conduct studies to confirm multiplication in this matrix so were unable to establish a role for almond oil as a reservoir. Furthermore, *S. capitis* NRCS-A continued to be isolated from Nantes Hospital NICU patients after removing the almond oil (9).

Foodborne *S. capitis* transmission in a hospital NICU could be via maternal breast milk, including expressed breast milk. In this case, the portal of entry is the mouth to the gastrointestinal tract of the neonate. While uncommon, bacteria such as *S. aureus* and *E. coli* can pass from expressed breast milk to the nose and throat of the infant and cause infection without causing maternal symptoms such as mastitis (84). *S. capitis* has been isolated from human breast milk (85). One study found that 8 (20%) of 40 breast milk samples contained *S. capitis* (85). This study showed that the *S. capitis* is capable

of persisting in breast milk (85). Therefore, if breast milk served as a foodborne vehicle for *S. capitis* transmission to neonates, the portal of entry would be the GIT. If the breast itself was colonised with *S. capitis* rather than the milk, the portal of entry would be the skin.

2.1.4 Hospital infection control

The Dunedin Hospital NICU follows standard precautions for infection prevention and control. The US Centers for Disease Control and Prevention (CDC) states that the standard precautions for patient care are common sense practices that protect the healthcare worker from infection, and prevent the spread of disease among patients (86). Standard precautions include performing hand hygiene, using personal protective equipment (PPE) if possible exposure to infectious agents, following respiratory hygiene, ensuring appropriate patient placement, properly handling and cleaning patient care equipment and devices, and handling textiles and laundry carefully (86). Transmission-based contact precautions include appropriate patient placement such as a single room for the affected patient, appropriate PPE including gloves and a gown for all patient contact, limiting movement of patients, disposable or dedicated patient equipment, and frequent cleaning and disinfecting (87). The Dunedin Hospital NICU does not follow transmission-based precautions for *S. capitis* as these focus on epidemics, while *S. capitis* is endemic in the NICU (personal communication, Roland Broadbent).

Hand washing is the basis of personal infection control in hospitals (88). As CONS live on the skin of humans, transmission is often through direct contact (as discussed in section 2.1.3). Removing infectious bacteria from the hands before touching patients reduces transmission (89). The World Health Organisation (WHO) recommends washing hands with soap when visibly soiled, or using an alcohol-based rub when not soiled (90). The WHO also recommends that hand hygiene is performed before and after touching a patient, before touching invasive devices regardless of wearing gloves, after touching an abiotic surface or objects around a patient, and after removing gloves (90). In a hospital intensive care unit, Conly et al. showed that hand washing protocols were not upheld by all medical staff (91). Before seeing patients as few as 14% of staff

washed their hands, whereas after seeing patients 28% were compliant with the protocol (91). Education programmes among staff in the ICU improved adherence to staff hand washing recommendations from before and after seeing patients to 73% and 81%, respectively (91). Nosocomial infections also dropped from 31% of patients to 12% after the education programmes (91). Antiseptics like chlorhexidine are sometimes used among neonates to prevent microorganisms from the skin entering the bloodstream during procedures (92). However, widespread use of antiseptics such as chlorhexidine can select for resistant CONS (92). In one study of neonates with bloodstream infections 31 (41%) of 51 CONS strains had reduced susceptibility, as measured by elevated minimum inhibitory concentrations (MICs), to at least one antiseptic regularly used in the NICU (92).

In the Dunedin NICU, hand washing with Microshield Skincare Cleanser (Schulker New Zealand Limited, New Zealand) is required by both parents and staff upon entering the NICU (93). Additionally, staff must wash their hands before and after patient or equipment contact, and before and after wearing gloves (94). Staff also must apply chlorhexidine 0.5% in 70% alcohol gel to their hands and forearms before performing any procedures (94). Gloves are worn for all patient contact. All equipment including stethoscopes and incubators are cleaned or sterilized between use (94). Stethoscopes are used for one neonate only, dismantled, and cleaned between neonates (94). Incubators are kept empty for as long as possible between neonates (94). Neonates requiring ongoing incubator care have their incubator cleaned weekly with Benzalkonium chloride solution 9.63g/L and Down to Earth dishwashing liquid (PZ Cussons, Auckland, New Zealand), and resistant staining or adherent marks are removed using De-solv-it solution (Vardon Industries, Victoria, Australia) (personal communication, Roland Broadbent).

2.2 Host defence in premature neonates

2.2.1 Neonatal skin

Skin is a physical barrier that defends the body from light, water loss, and irritants (95). Additionally, skin provides resilience to mechanical trauma, sensation, tactile discrimination, thermal regulation, acid mantle formation, and infection control (95). At birth, neonate's skin is already equipped with the ability to protect the infant during the change in environments from the fluid in the womb to the outside air (95). Soon after birth, the skin facilitates the neonate's colonisation with commensal organisms, while preventing the growth of harmful microorganisms (50).

Neonates are born with innate immune function, particularly within parts of the skin like the stratum corneum and the vernix caseosa (96). The stratum corneum is the outer layer of the epidermis that provides a protective air-liquid barrier (97). The stratum corneum contains host defense proteins called lysozyme and lactoferrin (96, 97). In addition, the stratum corneum has an acidic film called the acid mantle that facilitates the colonisation of commensal microorganisms, while preventing the growth of pathogenic bacteria (98, 99). At birth, a protective cream-like layer called the vernix caseosa covers the stratum corneum (98). The vernix is made up of 80% water, lipids, and proteins (100, 101). The vernix caseosa also contains lysozyme and other peptides associated with antimicrobial activity, and parasite inactivation (97, 99). The vernix may also facilitate the development of the acid mantle (97, 98).

The stratum corneum is established during the fourth month of gestation. However, it does not fully mature until the third trimester (102). Before maturation, the stratum corneum is deficient in structural proteins, making the skin thin and porous (95, 102). Neonates born before the end of the third trimester are susceptible to water loss, thermal instability, torn skin, and infection (95). In addition, premature neonates born before 28 weeks' gestation do not have a vernix caseosa (95). It takes approximately two to three weeks for the neonate's skin to mature to the same stage as term infants (102, 103). During this period, premature neonate's immature skin is at risk for infection. The lower the gestational age at birth, the greater the risk of high

transepidermal water loss (TEWL) (104, 105). TEWL measures the rate at which water crosses the skin layers into the environment (106). High TEWL scores indicate a poor skin integrity (107). Some NICUs use creams or emollients, such as sunflower seed oil, to cover the skin of premature neonates in an attempt to mimic the function of the vernix (107).

2.2.2 Other aspects of neonatal host defense

It is common for neonates admitted to NICUs to be of lower birthweight and lower gestational age (GA) than non-NICU neonates. Low GA is a major determinant of immune system immaturity (77). Examples of neonatal immune immaturity include decreased number and function of immune cells including monocytes, neutrophils, lymphocytes, and decreased production of cytokines and antibodies (77). An immature immune system means that neonates do not have adequate immune defenses to protect against infections (108). In addition, transplacental transfer of maternal antibody is lower among premature infants compared with those born at term (64). Stress and comorbidities further impair the neonate's ability to fight infection (109).

Premature neonates have decreased function of the gastrointestinal mucosa, including reduced barrier function, which results in increased permeability (110). For example, neonates born before 40 weeks (full term) have reduced numbers of gut epithelial cells including a specialized type of epithelial cell called the Paneth cell (111, 112). Paneth cells are important for host defense, shaping of the gut microflora, and development of gut epithelial cells (111, 113). Paneth cells secrete a protein called α-defensin that is important for host defense (111, 112). A study using rat models showed that rats without α-defensins had a different microbiota composition and were more likely to develop infections than the control group (112, 114). Therefore, the reduction in the number of Paneth cells likely contributes to the reduced immune function of premature neonates.

2.3 Neonatal bloodstream infections and sepsis

2.3.1 Epidemiology

CONS are the leading cause of healthcare-associated bacteremia (39) and sepsis in NICUs around the world (1-5). Bloodstream infections are the most common type of infections in NICUs (115). *S. capitis* pulsotype NRCS-A is now recognised as a major cause of bloodstream infections and sepsis in NICUs (9, 11, 15). In some NICUs where *S. capitis* NRCS-A is endemic, the bacteria colonise the skin of around a third of all neonates during their stay, and is associated with bloodstream infections in around a tenth of all neonates (16). A bloodstream infection is defined as bacteria in the blood, and is diagnosed by a positive blood culture (116). Historically CONS have been regarded purely as contaminants of clinical samples rather than as pathogens themselves (21). Although the role of CONS in bacteremia and sepsis has now been proven, there is still the possibility that the large number of cases reported is an over exaggeration due to contamination.

Low birthweight and low GA are both risk factors for bloodstream infections (5, 12, 117). Infants born <1500g, the definition of very low birthweight (VLBW), are more likely to develop a bloodstream infection during their hospital stay than those ≥1500g (115, 118). The combination of reduced immune function, increased likelihood of intravascular devices, and longer duration of hospital stay further increases the neonate's susceptibility to infections (118, 119). With advances in neonatal care, infants are surviving from lower GAs than ever before (5). The increased survival at low GA places more infants at risk of CONS infections.

Sepsis is a complication of a bloodstream infection. Sepsis is defined as the presence of an infection, such as a bloodstream infection, with the systemic inflammatory response syndrome (120). Sepsis is a severe immune reaction that can lead to organ failure and death (116). Sepsis criteria differ between neonates and adults, therefore adjusted criteria for sepsis diagnosis in neonates has been suggested to allow early identification, surveillance and epidemiologic studies (116). Haque et al. defines neonatal sepsis in patients if they have signs of an infection, and if they meet one or more of the seven

foetal inflammatory response syndrome (FIRS) criteria (116): tachypnoea (respiratory rate >60bpm), temperature instability (<36ºC or >37.9 ºC), capillary refill time of >3 seconds, white blood cell count (<4000 x10^9/L or >34,000 x10^9/L), C-reactive protein (>10pg/ml), IL-6 or IL-8 (>70pg/ml), and 16 sRNA gene PCR positive if PCR of the bacterial isolate is available (116). The presence of infection is often confirmed by blood culture. Due to the small circulating blood volume of neonates, the volume of blood collected from neonates is much smaller than from adults (116, 121). However, bacterial counts in the blood (CFU/ml) are much higher in neonates than adults so a smaller blood volume is required to achieve adequate sensitivity (122).

CONS are the most common type of bacteria isolated from premature neonates with LONS (12). The prevalence of sepsis in neonates is inversely proportional to the neonate's GA and birthweight (77). A 2002 study by Stoll et al investigated the relationship between sepsis prevalence and GA and birthweight, finding that of 6215 VLBW infants who survived more than three days, 1313 (21%) developed sepsis (12). The Stoll study also showed that of 656 neonates born at <25 weeks' gestation, 300 (46%) developed LONS (12). The risk of LONS decreased with increasing GA, and of 407 neonates born at >32 weeks' GA, only 10 (2%) developed LONS (12). Kaufman et al. (2004) showed that premature neonates who develop sepsis have a three times greater risk of death than healthy neonates (77).

2.3.2 Intravascular devices and bacterial translocation

A large proportion of neonatal bacteremia in NICUs is associated with intravascular catheters or other indwelling devices (12, 14) and the majority of these are caused by CONS (14, 123, 124). Intravascular catheters are the likely source of bloodstream infections when the bloodstream isolate is the same as the isolate from the catheter tip, skin site, or from blood drawn through the catheter (123-125). There are two predominant types of catheter-related bloodstream infection; those resulting from colonisation of the intravascular device, and those from contamination of the fluid that runs through the device (123). Contamination of the device occurs either from skin microorganisms colonising the catheter extra-luminally or contamination of the catheter

tip during insertion (123). If the tip is contaminated the bacteria enter the bloodstream intra-luminally (123).

Contamination of the catheter tip is often caused by biofilm-forming bacteria that adhere to the catheter then slough off into the host bloodstream (123). Bacteria that form biofilms account for approximately half of all intravenous-catheter-related bloodstream infections (118, 126). Although many neonates with intravenous catheters develop bloodstream infections, the catheter may not always be the source of infection. One study by de Brito et al (2009) found that the route by which the bacteria entered the bloodstream could not be identified in 70% of neonates, as the isolates from the blood did not match those isolated from the skin or the catheter (123). Another study by Valvano et al showed that the plasmid DNA from isolates from the catheter did not match that from blood isolates in three (43%) of seven patients with bacteremia (127).

As discussed in previous sections, the GIT of premature neonates is heavily and rapidly colonised by CONS (section 2.1.1), and neonates have abnormal mucosal permeability (section 2.2.2). Therefore, it has been hypothesised that bloodstream infections could be caused by CONS translocating across the gut barrier and into the blood (128, 129). In this view of pathogenesis, CONS translocate from the gastrointestinal tract to the mesenteric lymph nodes, and from there into the bloodstream (Figure 2). Bacterial translocation from the GIT to the mesenteric lymph nodes has been demonstrated in rats (130, 131). Soeorg et al. studied neonates with LONS to assess bacterial translocation from the GIT (13). Of the 22 neonates in the study, 21 (95.5%) experienced GIT colonisation by any species of CONS before they developed CONS sepsis (13). Eighteen (81.8%) of 22 neonates were colonised with the same species of CONS that caused sepsis (13). Analysis showed that 13 (59.0%) of 22 neonates with LONS had the same CONS species isolated from the GIT before the blood (13). Other studies have shown a similar pattern of CONS species that colonised the GIT before being isolated from the blood (128, 132). However, since neonates may be colonised at other sites by similar CONS strains, such studies do not prove causation.

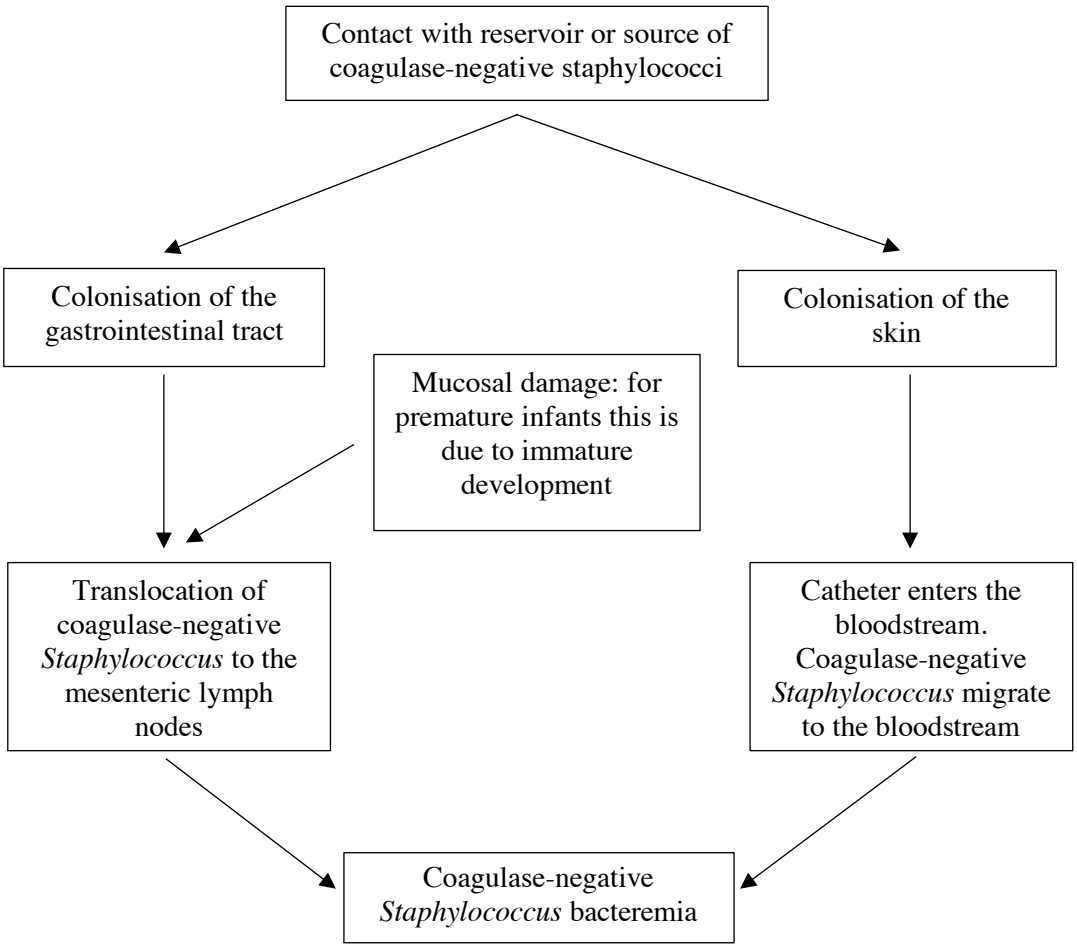

Figure 2: Hypothesised routes of CONS bacteremia.

Adapted from Costa et al, 2004

Colonisation prevention strategies should take into account the possibility that CONS bloodstream infections may originate from the skin or GIT. Hospitals have strict guidelines for intravascular catheter insertion to prevent infections (133). Such hospital guidelines include: choosing a skin site with low bacterial burden; using hand disinfection, a mask, gloves, cap and sterile drape; skin disinfection; handling and inserting the catheter with aseptic technique; and removing the catheter as soon as it is not needed (133). Preventing infection via bacterial translocation is difficult. Use of antimicrobials may make the situation worse by selecting for resistant CONS populations and damaging the microflora. Therefore, efforts should be targeted towards prevention of CONS colonisation at both the skin and the GIT. Studies on prevention should focus on identifying the source of colonisation then modifying its exposure among neonates.

2.4 *S. capitis* in the Dunedin Hospital NICU

S. capitis pulsotype NRCS-A has been isolated regularly from neonates in the Dunedin Hospital NICU since 2007, supporting the evidence that it is a strain capable of survival and persistance. Using whole genome sequencing (WGS), the *S. capitis* strain in the Dunedin Hospital NICU was shown to be the same clone found in other NICUs around the world (personal communication, James Ussher). However, the Dunedin Hospital strain exhibits some differences to the other strains, particularly the acquisition of a plasmid pSC16875, which is present in the majority of isolates (42/64), that contains genes for resistance to penicillin, tobramycin, fusidic acid, and reduced susceptibility to chlorhexidine (personal communication, James Ussher). The Dunedin Hospital strain also harbours the *ica*ADBC operon, which encodes genes that produce proteins essential for biofilm formation (personal communication, James Ussher). The Dunedin Hospital isolates were phenotypically resistant to amoxicillin and fusidic acid, and could produce biofilms (personal communication, James Ussher). In the Dunedin Hospital NICU alone, approximately one third of all neonates admitted to the unit have become colonised with this strain of *S. capitis* (personal communication, James Ussher). This puts a large proportion of neonates at risk for developing invasive *S. capitis* disease. In September 2013, a screening programme was implemented in the Dunedin Hospital NICU to identify colonised neonates and prevent transmission of *S. capitis*. All neonates in the Dunedin Hospital NICU were screened with axillary swabs weekly during their stay. In addition, environmental swabs were taken and *S. capitis* NRCS-A was isolated from stethoscopes and incubators (personal communication, James Ussher).

In December 2013, the NICU moved to a new location within Dunedin Hospital and all equipment was cleaned before this transition. Despite these measures, *S. capitis* recurred and persisted among neonates in the Dunedin Hospital NICU. Subsequently, anonymised voluntary screening of Dunedin Hospital NICU staff members was undertaken, and *S. capitis* NRCS-A was isolated from the hands of one staff member (personal communication, James Ussher). The only changes to hand hygiene practices, or other infection control procedures since 2013, was the implementation of wearing gloves for all direct contact with neonates, introduced in February 2017.

The Dunedin Hospital NICU screening programme for *S. capitis* is still active in 2018. However, currently only neonates born at less than 36 weeks' gestation are swabbed to identify colonisation in those most at risk of invasive infections. Although, *S. capitis* is regularly isolated from neonates, the hospital infection prevention and control team have not identified the reservoir, source, or mode of transmission. To our knowledge, no studies to date of *S. capitis* epidemiology in the Dunedin Hospital NICU setting have identified the reservoir, source, or mode of transmission, and few have proposed risk factors for colonisation (15, 16). Since understanding transmission is essential to design prevention and control measures, we sought to identify the risk factors for *S. capitis* colonisation of neonates in the Dunedin Hospital NICU.

3 Hypotheses and aims

Hypotheses

Our hypotheses for both the prospective and retrospective studies were based on the literature as summarised in our hypothesised chain of infection (Figure 1). There have been few studies investigating the risk factors for *S. capitis* NRCS-A colonisation among premature neonates (section 2.4), therefore our hypotheses were broad.

- We hypothesised that healthcare workers are sources of *S. capitis* NRCS-A.
- We hypothesised that antimicrobial exposure is a risk factor for *S. capitis* NRCS-A colonisation among premature neonates.
- We hypothesised that the NICU environment contains fomites which are sources of *S. capitis* NRCS-A.

Aim

The aim of this project was to identify the risk factors for *S. capitis* NRCS-A colonisation among premature neonates in the Dunedin Hospital NICU. The aim was investigated using both prospective study and retrospective studies.

4 Methods

4.1 Study site

Our study site was the NICU in Dunedin Hospital, New Zealand. Dunedin Hospital serves the Otago and Southland Regions, with an approximate catchment population of 315,000 residents (Statistics New Zealand, 2015). The Dunedin Hospital NICU has 16 rooms and 26 bed spaces, as shown on the floorplan in Figure 3. Approximately 68 clinical staff are employed by the NICU: 44 nursing staff, 10 consultants, and 14 registrars. Trainees including house officers, medical students, and student nurses rotate through the NICU. Allied health professionals include lactation specialists, radiographers, and speech language therapists. During the period from 1 September 2013 through 31 March 2015, 352 neonates were admitted to the NICU, and 236 were swabbed at least once (personal correspondence, Roland Broadbent). Of 236 neonates admitted, 58 (24.6%) were identified to develop *S. capitis* colonisation and of those, 4 (6.9%) developed an invasive infection (personal correspondence, James Ussher).

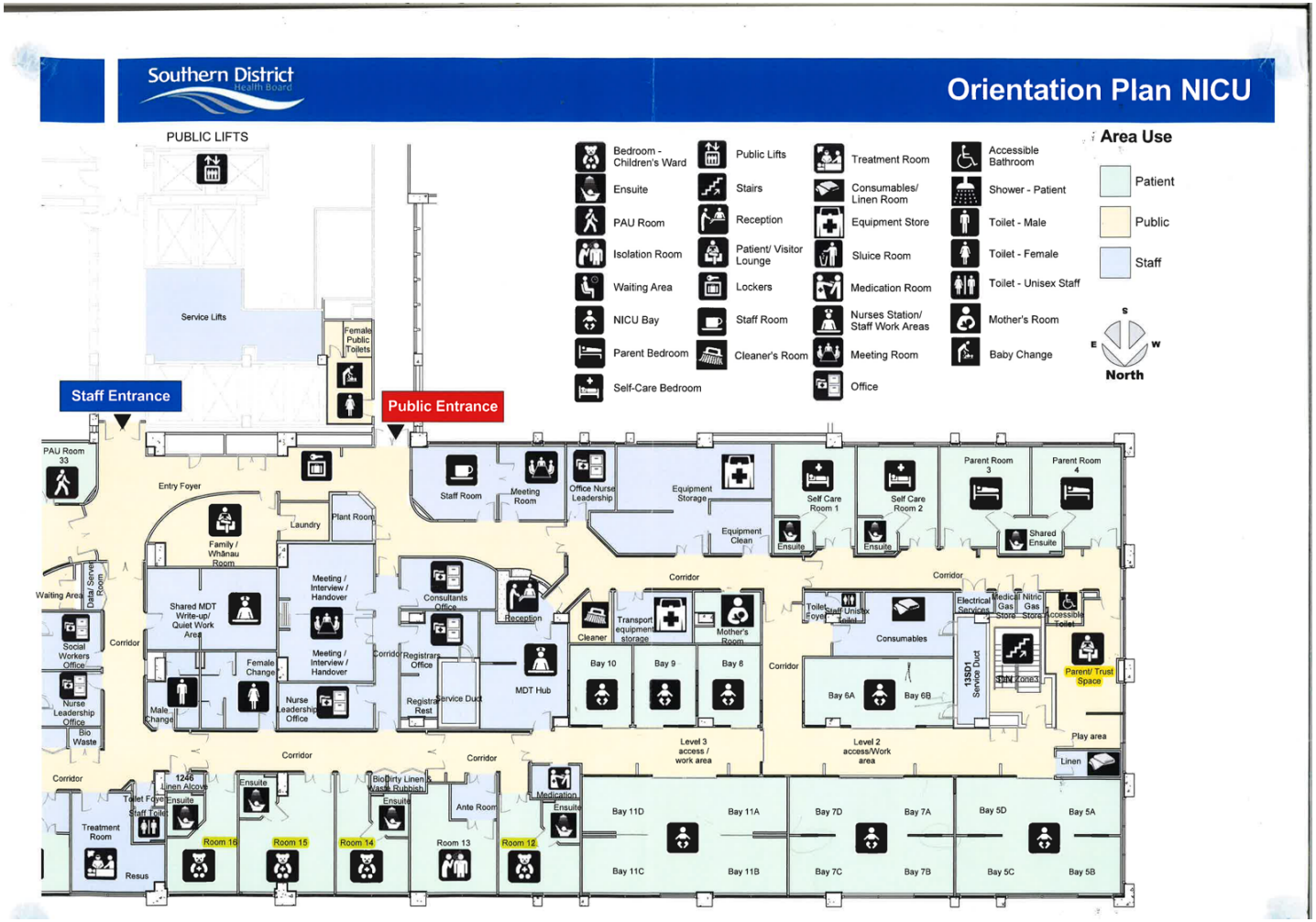

Figure 3: Floorplan of the neonatal intensive care unit, Dunedin Hospital, December 2013 through present

Yellow highlighted rooms – patient or public rooms not occupied by premature neonates

4.2 *S. capitis* surveillance among neonates

A surveillance programme seeking to identify NICU neonates with *S. capitis* colonisation was established in September 2013 in conjunction with the Dunedin Hospital Infection Prevention and Control Service. Colonisation status was sought by weekly axillary swab. From September 2013 through March 2015, all neonates were swabbed. From April 2015, testing was restricted to neonates born at < 32 weeks' gestation. Since neonates born at <32 weeks' gestation were at highest risk from invasive infection (from review of local data and personal communication, Roland Broadbent), they were targeted for surveillance. From July 2017, all neonates who were <34 weeks' gestation at birth were swabbed every Monday. In order to monitor *S. capitis* transmission among a larger proportion of neonates beyond those at greatest risk for invasive infection, from December 2017 surveillance was extended to neonates <36 weeks' gestation at birth. COPAN Transystem ™ (COPAN Italia, Brescia, Italy) sterile swabs were used to collect axillary swabs. Inoculated swabs were transported to the laboratory in the COPAN Transystem ™ capped tube containing Amies transport medium without charcoal.

Nursing staff selected one axilla per neonate and collected swabs using washed and gloved hands. The nurse gently wiped the neonate's entire uncleaned axilla in either a circular or stroking pattern. If any exudate was present, this was also collected on the swab. The nurse sent the swab in the transport tube labelled with body site of the swab, date, and neonate's identifying information to Southern Community Laboratories (SCL), Dunedin. All NICU neonates who met the gestational age criterion of the surveillance period were scheduled an axillary swab every Monday until either *S. capitis* was detected or the neonate was discharged from the NICU. *S. capitis* surveillance was not a study procedure, but we were able to access the weekly swab culture result to assign weekly participant *S. capitis* colonisation status.

4.3 Laboratory methods

SCL staff performed culture of axillary swabs, species identification and antimicrobial susceptibility testing of isolates. Swabs were plated on trypticase soy agar with 5% sheep blood and incubated in ambient air at 37°C overnight. Several colonial variants suspicious for *S. capitis* were chosen for identification based on varied size, opaqueness, smoothness, and clarity. Identification of *S. capitis* isolates was done using matrix associated laser desorption ionisation time of flight mass spectrometry (MALDI-ToF-MS) (Bruker Daltonics, Massachusetts, USA). Material from the colony resembling coagulase-negative *Staphylococcus* was placed on the polished steel target plate (Bruker Daltonics) and allowed to dry at room temperature. The dried spot was then overlaid with 1μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution (Bruker Daltonics) and allowed to dry for a further 15 minutes at room temperature. Mass spectra were generated with a Microflex LT, (Bruker Daltonics) using MALDI Biotyper™ automation control (Bruker Daltonics). Species identification was automatically performed using the Bruker Biotyper™ software and library. Isolates of *S. capitis* were confirmed as the NICU endemic clone by pulse-field gel electrophoresis at the Institute of Environmental Science and Research (ESR), Wellington, New Zealand.

After establishing that *S. capitis* pulsotype NRCS-A could be accurately distinguished from other *S. capitis* by the presence of the *mecA* gene (personal communication, James Ussher), from August 2017 preliminary identification of the endemic NICU clone was undertaken by phenotypic antimicrobial susceptibility testing. Disk diffusion testing according to standards and interpretive criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was used to detect resistance to cefoxitin as a marker of *mecA* gene presence (134). Results were reported to Health Connect South, an online database containing electronic clinical records. The NICU were notified of any cefoxitin-resistant *S. capitis* isolates.

4.4 Study design: prospective study

We conducted a prospective cohort study among admissions to the Dunedin Hospital NICU to determine the risk factors for *S. capitis* colonisation among premature neonates. Recruitment and data collection commenced on 1 July 2017 and is ongoing at the time of thesis submission. Data collected from 1 July 2017 through 28 February 2018 are described as a progress report in this thesis.

4.5 Study design: retrospective study

As we did not meet the sample size required for the prospective study, we investigated our study aim (section 3) by conducting a retrospective cohort study among admissions to the Dunedin Hospital NICU from 9 September 2013 through 9 March 2015. Extraction of retrospective data took place from 12 December 2017 through 16 February 2018.

4.6 NICU admissions and *S. capitis* colonised neonates

We collected the total number of admissions during both the retrospective and prospective studies. Juliet Manning provided these data from monthly admissions reports compiled by the NICU office administrator. We collected the number of admissions for the whole study period, and per month. We also collected the number of neonates colonised with *S. capitis*, and the number of neonates with invasive *S. capitis* infections during the study periods. Dr James Ussher provided these data from the Microsoft Excel spreadsheet of *S. capitis* positive swab results and from the laboratory information system.

4.7 Study population: prospective study

The prospective study population included all neonates <34 weeks' gestation at birth admitted to the Dunedin Hospital NICU and their birth mother if she was involved in the care of their child. Guardians other than the birth mother were not included as participants in the study.

Eligibility criteria

Inclusion criteria

- Neonates were eligible for enrollment if their admission to the NICU lasted for two or more weekly axillary swabs (8-14 days post-admission).

Exclusion criteria

- Neonates were not eligible for enrollment if their first axillary swab was positive for *S. capitis* pulsotype NRCS-A.

4.8 Study population: retrospective study

The study population included all neonates admitted to the Dunedin Hospital NICU, who had at least one axillary swab from 9 September 2013 through 9 March 2015.

Eligibility criteria

Inclusion criteria

- Neonates were eligible for enrollment if their admission to the NICU lasted for two or more weekly axillary swabs (8-14 days post-admission).

Exclusion criteria

- Neonate's first axillary swab was positive for *S. capitis* pulsotype NRCS-A.
- Neonates admitted to the NICU before the surveillance began.
- Neonates swabbed more than once but did not receive their scheduled second swab.

4.9 Sample size determination

Based on number of neonates admitted in the Dunedin Hospital NICU in previous years, for both the prospective and retrospective studies we expect to have a sample size of about 75 neonates over a 6-month period that meet our inclusion criteria (remain in the unit for at least two rounds of swabbing). We expect about 38% to become colonised, therefore, 29 colonised neonates over a 6-month period. With this number of cases, we will have 80% power to detect OR of 3.6 if the prevalence of the risk factor is 0.4 and OR of 5 if the prevalence of exposure is 0.1. If there is a single risk factor we anticipate the OR to be large, however the study will be underpowered to detect multiple risk factors with smaller effects.

4.10 Definitions

Colonised neonate definition: an individual neonate that ever had a positive *S. capitis* swab result.

Non-colonised neonate definition: an individual neonate that never had a positive *S. capitis* swab result.

Colonised and non-colonised neonates were categorised for each exposure week of their stay in the NICU as a case or a control for that week. The exposure week was an arbitrary period a week prior to a positive or negative *S. capitis* swab result. The exposure week was measured from 6am Monday to 5.59am the following Monday. Axillary swab results on both the Monday of, and the Monday following, the exposure week were necessary to determine if the neonate's exposure week was a case or a control. The exposure weeks and swabbing pattern are shown in Figure 4.

Case definition: A week in which an eligible neonate had a negative swab result on the starting Monday of the exposure week, and a positive swab result on the following Monday.

Control definition: A week in which an eligible neonate had a negative swab result on the starting Monday of the exposure week, and a negative swab result on the following Monday.

Cases and controls were included in a nested case-control analysis. The same eligible neonate could contribute both case and control data to the nested case-control analysis. Eligible neonates contributed control data until they became a case, were discharged from the NICU, or missed a swab. Eligible neonates could only contribute case data once, after which we stopped collecting data for the neonate.

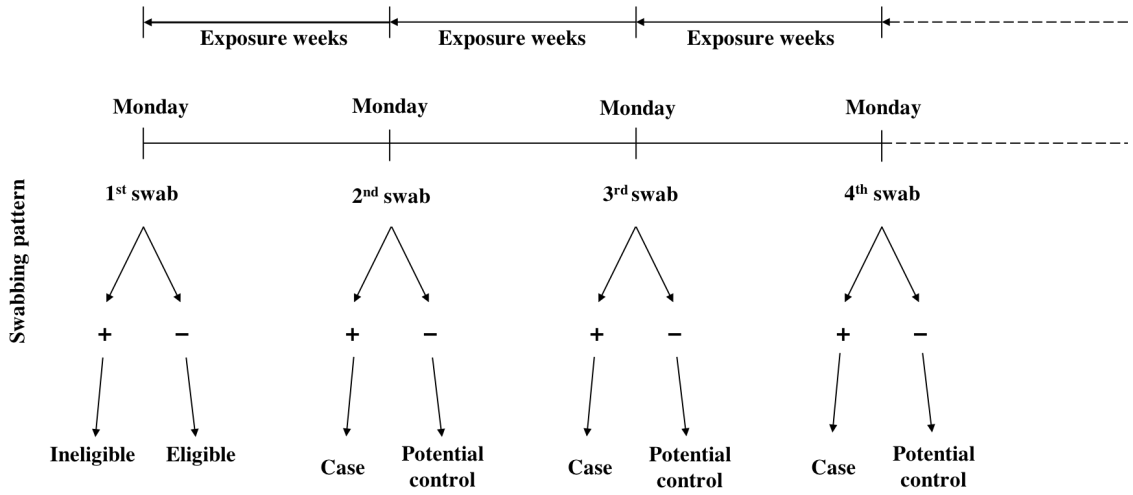

Figure 4: Study eligibility and data collection process, prospective and retrospective *S. capitis* risk factor studies, Dunedin Hospital NICU, 2013-2018

+ - positive; – - negative; NICU – neonatal intensive care unit.
Swabs and CRFs continued every week until the neonate missed a swab, had a positive *S. capitis* test result, or was discharged from the NICU.

4.11 Recruitment: prospective study

We recruited neonates and their birth mothers from among Dunedin Hospital NICU admissions throughout the prospective study as they became eligible. Consent for participation in the study was sought after receiving a negative result from the first swab, and if the neonate was expected to remain in the NICU through the next Monday, ascertained by nursing or medical staff. NICU clinical staff recruited all neonate and birth mother participants. Recruitment included the issuing of forms to eligible participants, witness of signature on the consent form, and answering family queries.

4.12 Participants: retrospective study

I identified neonates for participation in our retrospective study using results from the *S. capitis* surveillance programme during the study period, and their Dunedin Hospital medical records. If the neonate met the eligibility criteria (section 4.8) they were included as a participant in the retrospective study, there was no contact with neonates, or their parents or guardians.

4.13 Data collection: prospective study

4.13.1 Case report forms

We sought validated and widely used questionnaire instruments from past studies of staphylococcal transmission and disease in NICU settings. We obtained questionnaires from the Prevention and Response Branch, Division of Healthcare Quality Promotion, US Centers for Disease Control and Prevention. The questionnaires were used for epidemiologic studies of a range of pathogens, including *Staphylococcus* spp. Following a review of *S. capitis* literature, I adapted these questionnaires to address the hypotheses of our study. I designed protocol-driven case report forms (CRFs) for weekly data collection by clinical staff. I created three types of CRFs: 1) the *S. capitis* test result CRF (Appendix 1); 2) the baseline CRF (Appendix 2); and 3) the weekly CRF (Appendix 3). With the close involvement of Associate Professor Roland Broadbent and NICU charge nurse Juliet Manning, CRFs were reviewed, piloted, and edited prior to the initiation of our study. Juliet Manning was the NICU staff member with primary responsibility for overseeing data collection. Three clinical staff members were trained for data collection, including a specialised research nurse and two other nurses who were interested in the research. Juliet Manning trained the staff by reviewing every question in each form with the trainees, and instructing them on the data sources for each section. I created a manual of CRF guidelines (Appendix 4) to explain explicitly how to ask questions and record responses. After piloting, the CRF guidelines were incorporated into the CRFs as instructions at the start of each section and each question. Additionally, I reviewed the first CRF with the staff member after data collection to ensure accurate completion.

***S. capitis* swab result CRF**

I created the *S. capitis* swab results CRF (Appendix 1) to collect the result from the weekly *S. capitis* swab test. The form included the participant identification number, the date of the swab test and the result: negative or positive for *S. capitis*. A NICU clinical staff member completed the CRF when the swab test results were made available on the online Health Connect South database.

Baseline CRF

The baseline CRF (Appendix 2) collected information for both the neonate and their mother in the domains listed below:

- Infant demographics including sex, date of birth, date of admission, gestational age, birthweight, ethnicity, and number of siblings.
- Information about the birth including delivery type, devices used during the birth (e.g., forceps), multiple births, where the birth took place, and the type of facility.
- Maternal demographics including date of birth and ethnicity.
- Maternal antimicrobial use during pregnancy.
- The pregnancy and parturition history including events such as cerclage, group B *Streptococcus* colonisation, and premature rupture of membranes.

The data in the baseline CRF form were collected from the neonate's admission forms, and by interview of the birth mother. The baseline CRF was completed after consent was obtained, but before data collection for the first weekly CRF. If the birth mother was not involved in their child's care or did not give consent for her own participation in the study, we did not collect data regarding the mother's demographics, antimicrobial use, or history of the pregnancy and parturition.

Weekly CRF

The weekly CRF (Appendix 3) covered the exposure week: a 7-day period from 6am Monday to 5.59am the following Monday. These times were chosen to match the approximate time the swab tests were taken each Mondays. Domains of data collected in the weekly CRF included:

- Infant comorbidities, procedures, and devices.
- Antimicrobial use, and other medications including injectables.
- Parental contact, and contact with other neonates (e.g. twin).
- Enteral feedings including breast milk feedings, and formula feedings.

- Environment such as bed space number, incubator number, cot number, stethoscope number, infant weight, equipment use, number of cares, and equipment used during cares.
- Other procedures such as apnoeas requiring stimulation, other nursing procedures (e.g., urine collection), or other procedures by allied health professionals (e.g., head ultrasound scans).
- Consultant and registrar contact including the staff member title ('consultant' or 'registrar'), the shift time, the type of examination or procedure, and the duration of the contact.
- Nurse contact including the nurse code, the shift time, and the details of any other neonates being cared for by the same nurse on the same shift. The details of other neonates included bed space number and *S. capitis* status: positive, negative, or unknown.
- The condition of the skin on the birth mother's breast.

The trained NICU clinical staff members collected the data for the weekly CRF. The data were collected from the medical records for each neonate. Incubator, cot, and stethoscope numbers were recorded from either equipment serial numbers or identification numbers attached to the equipment by staff members. The last section of the weekly CRF collected maternal data regarding the skin on the mother's breast, and were sought by interview of the birth mother. The clinical staff completed this section at the start of the week to reduce recall error. Additionally, we recorded neonate contact of staff members in the weekly CRF. We used the term 'contact' to mean either direct physical contact with the neonate by the staff member or contact with equipment, such as stethoscopes, at least once during their shift or examination. We collected data on nurses, consultants, and registrars who routinely worked in the NICU. No data were collected on trainees or allied health professions as their shifts were not routine, and did not regularly require contact with the neonates. Roland Broadbent or Juliet Manning assigned the staff members a code so that specific staff members could not be identified. Roland Broadbent supplied the consultant and registrar timetables, so the staff code could be matched to the 'consultant' or 'registrar' recorded on the CRF.

The weekly CRF was completed at the end of the exposure week. The first exposure week from which we sought data was the week between the first and second axillary swab, which is shown in Figure 4. The data for the first CRF were collected on the Monday or Tuesday after the exposure week, following informed consent and completion of the baseline CRF. This continued each week for as long as the neonate remained eligible for weekly surveillance. For neonates with a positive swab result, the final CRF captured information from the exposure week before the *S. capitis* positive swab. If the neonate remained *S. capitis* negative, the last CRF captured information from the exposure week before the negative swab. If the participant was discharged from the unit between swabs, we did not collect data from the partial week.

4.14 Data collection: retrospective study

4.14.1 Baseline data

We modified the baseline CRF from the prospective study (Appendix 2) for the purpose of the retrospective study (Appendix 5). The changes to the baseline CRF included:

- Added eligibility section including eligibility yes or no; swab result if not eligible, and if the swab was positive for *S. capitis*, date of the positive swab.
- Added availability of weekly data section including availability yes or no. If the neonate was eligible but data was not available we collected the number of weeks of data lost, swab result, and the date of the positive swab if positive for *S. capitis*.
- Added section on the neonates stay in the NICU including date of admission, date of discharge, if the neonate was alive or deceased at discharge, and reason for discharge.
- Removed all sections in the prospective CRF regarding the mother. The mother was not included in the retrospective study as we did not have ethical approval to access to the mother's medical records.

In order to collect data for the whole study population, the baseline CRF was completed for all neonates, irrespective of eligibility status. The birth record that formed part of the neonate's medical record was a source document for the neonate's demographic information such as sex, gestational age, birthweight, delivery data, and birthplace. If the neonate was not born in Dunedin Hospital, a NICU admission form was completed by nursing staff upon the neonate's admission and was a source document for the same data as the birth record. The free-form Dunedin Hospital NICU discharge letter, written by a registrar, was a source document for neonate demographic information including sex, gestational age, birthweight, date of admission, and date of discharge. The Southern District Health Board NICU care plan booklet is supplied for every neonate admitted to the NICU and is completed by nursing staff. The NICU care plan booklet was a source document for sex, ethnicity, gestational age, birthweight, delivery type, and family information. If the data were discordant between the sources, the birth record data was used as the main source, or if unavailable the NICU admission form was used. The data for the axillary swab test was extracted from a Microsoft Excel spreadsheet (Microsoft Corporation, Washington, USA) of test results provided by Dr James Ussher. When medical records were unavailable, data were collected from the online copy of the neonate's discharge letter through the Health Connect South online database. Health Connect South was also a source for *S. capitis* surveillance swab test results for all neonates.

4.14.2 Weekly data

We modified the weekly CRF (Appendix 3) from the prospective study for the purpose of the retrospective study (Appendix 6). After communication with Roland Broadbent and Juliet Manning regarding the accuracy of the data within the neonate's medical records, several sections were removed or altered as follows:

- We removed parental and family contact sections, as not all staff recorded the type of contact and duration of contact consistently.
- Enteral feeding data sought changed to total number of feeds, type, and route in the last 7 days from of type, route, and duration for all feeds within 24 hours as

the latter was not consistently recorded and was thought unlikely to contribute additionally to the quality of data.

- We removed incubator, cot, stethoscope numbers, and equipment as the identification numbers were not recorded in the medical records.
- We removed neonate cares, and other nursing procedures as it was not consistently recorded and was thought unlikely to contribute additionally to the quality of data.
- Consultant contact, registrar contact, and nurse contact were removed as we did not have consent to trace staff contact, and contact was not reliably recorded in the medical records.
- Apnoea requiring stimulation data sought was changed to the period of the last 7 days from the last 24 hours. Apnoeas data were contained on a structured sheet, including the date, the apnoea event, and the stimulation required, therefore were likely to be accurate.

As with the baseline CRF, we included a section for the swab test result for the exposure week, and the date of the axillary swab collection. We collected information in the weekly CRF for eligible neonates only. The data sources were the neonate's medical records, the Microsoft Excel spreadsheet of *S. capitis* positive swab results, and Health Connect South. We used the nurse, consultant, registrar, and allied health professional staff entries in the neonate's medical record to collect information on medical history, NICU procedures, and external procedures (e.g., head ultrasound scans). NICU category 4 or 5 charts are daily medical charts filled out by the nursing staff: category 5 are double-sided charts for the most intensive neonates, and category 4 are one-sided charts for the less intensive neonates. The category 4 and 5 charts were used as the source documents for information for medical history, NICU procedures, enteral feeds, bed space number, and if the neonate occupied an incubator or a cot.

Information on NICU procedures were sought from record sheets for laboratory tests, blood transfusions, phototherapy, and retinopathy of prematurity. Medication sheets that contained medication administration details were part of the neonate's medical records and were the source for data on the use of antimicrobials, other medications, and injectables. Neonate's weight was sourced from a weight form, which contained

weight, the date, and a freehand growth plot. Data on apnoeas requiring stimulation were collected from a sheet that contained details on the date, apnoea or bradycardia event, and the intervention required. Data on external procedures, if not found in the nursing entries, were collected from copies of the procedure test results. The test results were loose sheets in the medical records that contained the date, type of test, and the results.

As with the prospective study, the retrospective weekly CRF was completed for the exposure week. The first exposure week was 6am Monday of the neonate's first swab to 5.59am Monday of their second swab. This continued until the neonate missed a swab, had a positive *S. capitis* swab result, or was discharged from the NICU. The retrospective weekly CRF was completed upon receipt of the medical notes and after completion of the baseline CRF. The weekly CRFs were completed in chronological order for each neonate.

4.15 Data management: prospective study

We developed codes for all clinical staff in regular contact with neonate participants. Associate Professor Roland Broadbent supplied the codes and shift timetables for the consultants and registrars and charge nurse of the NICU, Juliet Manning, supplied the nurse codes. I created another set of codes to ensure it was not possible to trace the supplied codes back to the individual staff members. To create the consultant and registrar codes I used C or R, respectively, followed by a randomly generated number (e.g., R07). To create the nurse codes, two unique letters (e.g., IL) were randomly generated for each staff member. No randomly generated letter pairs matched the codes provided by Juliet Manning. I kept a Microsoft Word document (Microsoft Corporation, Washington, USA) with the link between the codes on a password protected computer in the Centre for International Health (CIH) offices.

Trained NICU staff completed paper CRFs. Juliet Manning notified me once forms were completed, and I collected the forms from the NICU. I entered the data into a study database designed using the University of Otago REDCap database (service version 8.2.0, Vanderbilt University, Tennessee, USA) on a password protected

computer at the CIH offices. I reviewed forms for completeness and accuracy. If any data were missing or unclear, I contacted the clinical staff member who completed the forms for a response and personally amended the forms based on their feedback. The CRFs were stored in a locked filing cabinet in the CIH offices for the remainder of the study.

Once the progress report of the prospective study is complete, the documents with the link between the staff codes will be transferred from the computer in the CIH offices to a password protected computer in Juliet Manning's office in the Dunedin Hospital NICU. The CRFs from March 2018 onwards will be stored in a locked file cabinet in the NICU offices until the prospective study is complete. Additionally, access to the REDCap database was transferred to Juliet Manning and Roland Broadbent for the remainder of the prospective study.

4.16 Data management: retrospective study

The Microsoft Excel spreadsheet (Microsoft Corporation, Washington, USA) of test results provided by Dr James Ussher included the neonate's unique National Health Index (NHI) identification number. From the Excel spreadsheet, I made a list of all neonates who had been swabbed at least once during the retrospective study period. Using the neonate's NHIs, Associate Professor Roland Broadbent ordered the notes from the clinical records team in Dunedin Hospital. We ordered 20-30 records per week. For each weekly batch of notes, the Dunedin Hospital clinical records team provided a sheet of paper with the NHIs of each neonate in the batch. The sheet was marked next to each NHI if the records were unavailable. I assigned each neonate a retrospective study ID, starting at R001, R002, and so on. In the retrospective study, I did not collect data on paper CRFs but instead entered the data directly into the REDCap online database on a password protected computer at the offices of the Women's and Children's Health Department. I created a Microsoft Word (Microsoft Corporation, Washington, USA) participant identification document on a password protected computer at the CIH offices, that matched the study ID, and the NHI. If any data were missing from our database after data collection, I was able to trace the study

number to the neonate and find the missing information. The participant identification document was deleted when statistical analysis was completed.

4.17 Statistical analysis

Data were downloaded from the University of Otago REDCap service version 8.2.0 (© Vanderbilt University, Tennessee, USA) into Stata/IC version 15.1 (StatCorp, College Station, TX, USA). Data on Dunedin Hospital NICU admissions and *S. capitis* colonised neonates were provided by Juliet Manning and James Ussher on a Microsoft Excel spreadsheet (Microsoft Corporation, Washington, USA) that were imported into Stata/IC. Datasets for the prospective study included test result CRFs, baseline CRFs, and weekly CRFs. Data sets for the retrospective study included baseline data and weekly data.

4.17.1 NICU admissions and *S. capitis* colonised neonates

Data on Dunedin Hospital NICU admissions and *S. capitis* colonised neonates were used to create epidemic curves for the both the retrospective and prospective study periods. The epidemic curves show the total number of admissions compared to the number of neonates with *S. capitis* positive swab results per month during the study periods. The incidence rate (cases per admission months) for the prospective study was compared the incidence rate for the retrospective study using a Poisson regression model.

4.17.2 Prospective study

Describing the prospective study population

We used baseline data to describe the characteristics of the participants including both colonised neonates and non-colonised neonates from our prospective study progress report. Test result data was used to classify participants as colonised neonates or non-colonised neonates. Descriptions of the baseline data for each group were presented as numbers and proportions for categorical or binary variables, or means and standard deviations (sd) for continuous variables.

Once the prospective study is complete, we will perform a nested case-control analysis using conditional logistic regression. This analysis is not part of this thesis.

4.17.3 Retrospective study

Describing the retrospective study population

We used baseline data to describe the characteristics of our study population. In order to assess selection bias we compared i) total eligible neonates and total ineligible neonates; ii) eligible colonised neonates and eligible non-colonised neonates; and iii) eligible neonates with available weekly data and eligible neonates without available weekly data. Descriptions of the baseline data for each group, and the total, were presented as numbers and proportions for categorical or binary variables, or means and standard deviations (sd) for continuous variables.

Eligible neonates with available data

Eligible neonates with available weekly data were used to investigate *S. capitis* risk factors. We first identified and labelled the exposure weeks for each infant as either case or control based on our case and control definitions. To control for the hypothesised patterns of *S. capitis* cases in the Dunedin Hospital NICU over time, we matched controls to the case exposure week on calendar time using modified risk-set matching. The modification was necessary to obtain a sufficient number of matched controls. For each case week, we included all control weeks for the four weeks previous to the case's positive swab. An individual neonate could provide multiple weeks of control data per case, for as many cases as applicable. Individual neonates could provide both case and control data. Additionally, an individual neonate could have their control data matched to their case data. The cases and their controls were assigned a set number for grouping purposes. All sets were merged into a matched case-control dataset. Baseline data were then merged to the dataset based on retrospective study ID number. The final dataset included cases, controls matched to case exposure week and three weeks previous, and baseline data.

Among the neonates who provided case data, control data, or both, we identified the individual colonised and non-colonised neonates. We used baseline data to describe their characteristics, antimicrobial use, and inflamed skin over the neonate's entire stay in the NICU. Numbers and proportions were presented as described for the retrospective study population.

The baseline and weekly data in the matched dataset were grouped into sections including: baseline data, antimicrobials, other medications, medical history, procedures and devices, other procedures, enteral feedings, weight, type of bed, bed space numbers, and room numbers. Numbers and proportions and means and sd were presented as described above for each section. For unadjusted and adjusted analyses we used conditional logistic regression, to estimate the matched odds ratios, 95% confidence intervals and p-values. We used robust standard errors to account for correlations between repeat measures on the same neonate within a matched set. The set number was used to group the matched cases and controls. Unadjusted conditional logistic regression was performed on all variables that had three or more observations in each exposure category and had data for both cases and controls. If variables with too few data points were included, the estimates were too small to interpret or the model did not converge so no estimates were available.

The sample size was too small to do a full multivariable analysis, so I adjusted the analysis for two main known confounders in addition to calendar week. Low GA, low birthweight, and length of stay in the NICU are all strongly associated with increased risk of CONS infections among neonates (12). Low GA at birth, low birthweight, and length of stay were also associated with risk of *S. capitis* colonisation among neonates in the Dunedin Hospital NICU (personal communication, James Ussher and Roland Broadbent), therefore likely to be confounders in our analysis. I adjusted for GA, and then for both GA at birth and length of stay. I did not include birthweight as birthweight and GA at birth were highly correlated in our study, therefore confounding was likely explained by GA. We chose GA at birth instead of birthweight as many of the treatments in the NICU are based on GA rather than birthweight. We used categorical variables of GA and length of stay for the adjusted analysis as we thought a linear relationship between GA and length of stay and outcome was unlikely, and there was

not sufficient data to model the relationship more accurately. GA was categorised into ≤32 weeks and >32 weeks as neonates born at ≤32 weeks' gestation is the definition of very preterm (135). Length of stay was arbitrarily set at ≤40 days and >40 days, as 40 days was the mean length of stay and there was not sufficient data to create more than two categories.

The adjusted conditional logistic regression models were fit for all exposure variables with ten or more observations in each exposure level, and data for both cases and controls. As with the unadjusted analysis, if too few data points were included in the analysis, the estimates were too small to interpret or the model did not converge so no estimates were available.

ORs, 95% CIs and p-values for all variables with enough data for adjusted analyses were presented in a table which included unadjusted analyses, analyses adjusted for GA, and analyses adjusted for GA and length of stay.

In order to examine patterns in location of cases over calendar time, I created a study week number for each week of the retrospective study. Our study began on Monday 9 September 2013, therefore the week from Monday 9 September through Monday 16 September was assigned as study week 0. Our study ended the week of 23 March 2015 through 30 March 2015, which was assigned as study week 83. Study weeks were used for a diagram of the NICU to show the bed space and room in which neonates became positive for *S. capitis* over time. The number of *S. capitis* positive neonates per study neonate week per room was also included in the case bed space diagram as a measure of incidence. Incidence was calculated as the number of cases over the number of controls per room over the study period. Study weeks were also included in a diagram of the cases born as a part of a multiple birth to show the number of weeks between twins becoming positive for *S. capitis*.

4.18 Consent, ethics, and funding: prospective study

Consent

I designed a participant consent form (Appendix 7) to record consent. Additionally, I created a participant information sheet (PIS) (Appendix 8) to inform the parents or guardians of the objectives of the study and the details of their participation. The neonate's participation was not dependent on the birth mother's participation. I collected the consent forms from the Dunedin Hospital NICU office. Consent forms were secured in a locked file cabinet in the CIH offices.

Ethics

We obtained ethics approval from the New Zealand Health and Disability Ethics Committee via the full review pathway (reference number 17/NTB/59). A copy of the approval letter is included in Appendix 9. Additionally, we attained Locality Authorisation from Health Research South (Appendix 10) for our study to proceed in Dunedin Hospital NICU (project ID: 01343). This process was aided by Associate Professor Roland Broadbent. We sought Maori Consultation from the Ngai Tahu Research Consultation Committee (Appendix 11).

Funding

I was provided with office space in the CIH offices and a computer (loaded with Stata/IC software for data analysis) by the Department of Preventive and Social Medicine, University of Otago. Data collection was hosted by staff of the Dunedin Hospital NICU who provided support for participant consent and enrollment and resources for study materials. Southern Community Laboratories provided *S. capitis* surveillance testing of neonates as part of an ongoing infection prevention and control programme.

4.19 Ethics and funding: retrospective study

Ethics

Ethics approval was obtained from the University of Otago Human Ethics Committee (Health) via the 'Minimal Risk Health Research – Audit and Audit related studies' pathway (reference number HD16/050). A copy of the approval letter is included (Appendix 12). The study also had locality authorisation from Health Research South (Appendix 13) for the study to take place in Dunedin Hospital (project ID: 01282). Ethics approval and locality authorisation were obtained by Dr. James Ussher and Associate Professor Roland Broadbent. I was added to the approvals so that I was able to undertake the study.

Funding

I was provided with office space in the Women's and Children's Health Department in the Children's Pavilion in Dunedin Hospital. Medical records were provided by the clinical records team at Dunedin Hospital.

5 Results

5.1 NICU admissions and *S. capitis* colonised neonates

The number of admissions and number of neonates colonised with *S. capitis* per month during the 2013-15 retrospective study period and the 2017-18 prospective study period are shown in Figure 5. Of the 58 neonates colonized with *S. capitis* during the retrospective study, 46 were born ≥32 weeks' gestational age, 10 were born ≥34 weeks' gestational age, and 2 were born ≥36 weeks' gestational age.

The incidence rate of cases per admission over the retrospective study period was 16 per 100 admissions. The incidence rate of cases per admission over prospective study period was 3 per 100 admissions. The incidence rate during the retrospective study period was 5.2 (95% CI 2.3-10.8) times higher than the incidence rate during the prospective study period ($p<0.001$).

A progress report from the ongoing prospective study is presented at the end of the Results chapter. The data collection for the retrospective study is complete and a full analysis of this study in presented in the following sections.

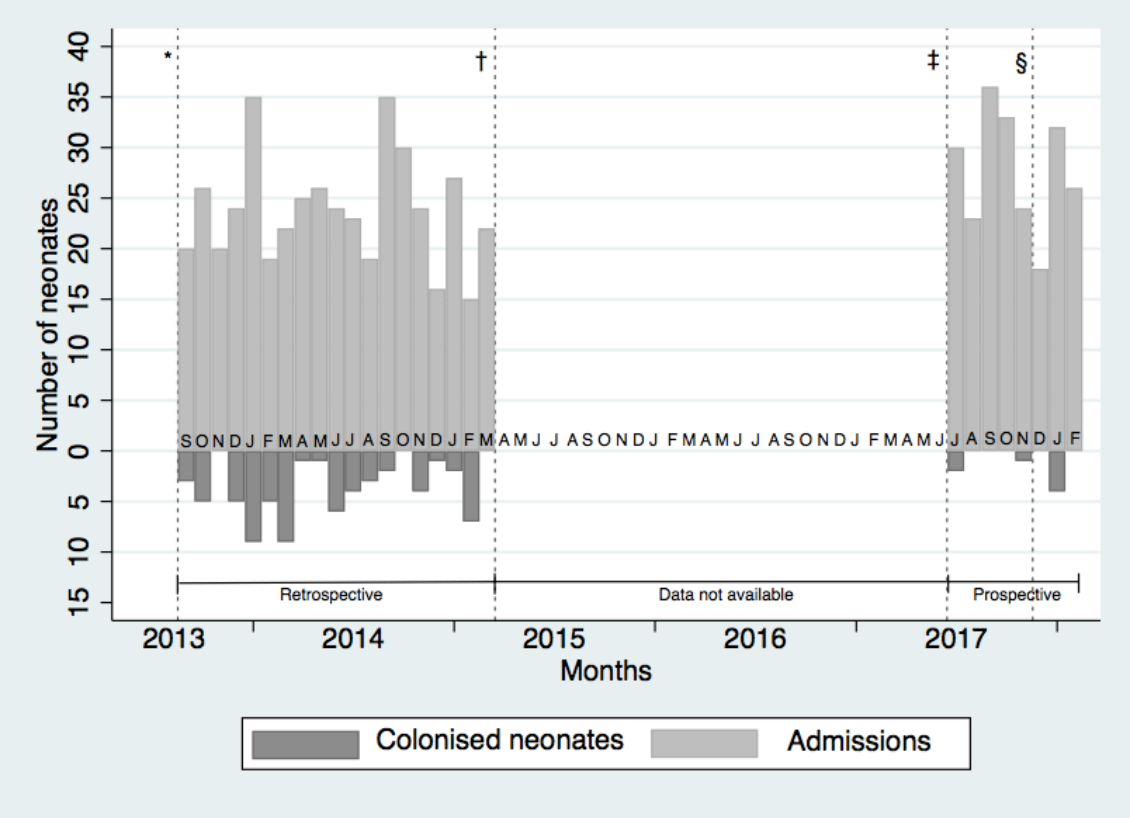

Figure 5: Epidemic curve of the admissions and neonates colonised with *S. capitis*, Dunedin Hospital neonatal intensive care unit, September 2013 through February 2018

Months of the year are marked with their initial; Retrospective study period – September 2013 through March 2015; Prospective study period – July 2017 through February 2018.

* *S. capitis* swab tests for all neonates began September 2013.
† *S. capitis* swab tests restricted to neonates <32 weeks gestational age, April 2015.
‡ *S. capitis* swab tests extended to neonates <34 weeks gestational age, July 2017.
§ *S. capitis* swab tests extended to neonates <36 weeks gestational age, December 2017.

5.2 The retrospective study population

Of 352 neonates admitted to the Dunedin Hospital NICU September 2013 through March 2015, 236 (66.3%) neonates were swabbed at least once during their stay and of these 117 (49.6%) were eligible for participation. Of 119 (50.4%) ineligible neonates, 88 (73.3%) were swabbed once, 16 (13.3%) had a positive first swab none of which were readmissions, 9 (7.5%) were admitted to the Dunedin Hospital NICU before the study began, and 6 (5.0%) did not receive their scheduled second swab. The flow diagram of admissions and their eligibility for inclusion is shown in Figure 6.

Of 117 eligible neonates, medical records were available for 64 (54.7%). Of 64 neonates with medical records, 26 (40.6%) were of neonates colonised with *S. capitis* and 38 (59.4%) were of neonates not colonised with *S. capitis*. Of 53 (45.3%) neonates with unavailable medical records, 16 (30.2%) were of neonates colonised with *S. capitis* and 37 (69.8%) were of neonates not colonised with *S. capitis*. All unavailable medical records were due to asbestos contamination of medical record storage rooms in Dunedin Hospital preventing safe access and use (136).

Of the 58 neonates that were colonised with *S. capitis* NRCS-A during the retrospective study period, 4 (6.9%) developed an invasive infection.

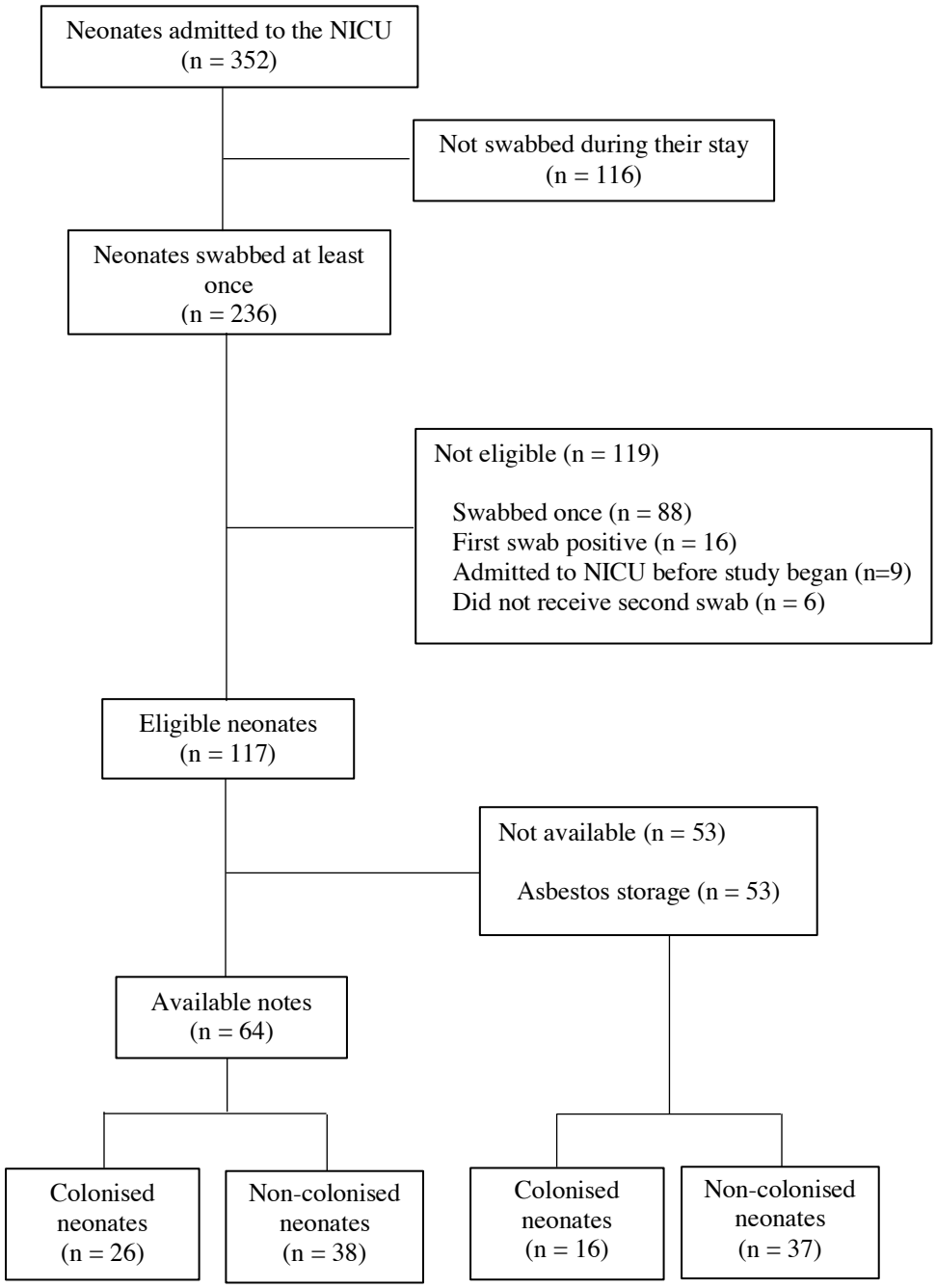

Figure 6: Flow diagram of the neonates and their eligibility for inclusion in the retrospective study, Dunedin Hospital NICU, September 2013 through March 2015

NICU – neonatal intensive care unit

5.2.1 Eligible and ineligible neonates

Table 2 shows the baseline characteristics of all 236 neonates who were swabbed at least once during their stay in the NICU, including 117 neonates who were eligible for the study, and 119 neonates who were ineligible. Among the 117 eligible neonates, 73 (62.4%) were New Zealand European compared to 87 (73.1%) among the 119 ineligible neonates. Twenty-four (20.5%) among the eligible neonates were Māori, compared to 10 (8.4%) among the ineligible neonates. The mean (standard deviation) gestational age at birth was 33.0 (± 0.4) weeks for eligible neonates and 35.7 (± 0.4) weeks for ineligible neonates. The mean (standard deviation) birthweight was 1,981 (± 77.7) g for eligible neonates and 2,667 (± 89.9) g for ineligible neonates. Among the eligible neonates, 31 (26.5%) were born as a part of a multiple birth, compared to 21 (17.7%) among ineligible neonates. The mean (standard deviation) length of stay was 30.5 (± 2.0) days for eligible neonates and 14.7 (± 1.9) days for ineligible neonates.

Table 2: Baseline characteristics of participants swabbed at least once by eligibility status, Dunedin Hospital NICU, September 2013 through March 2015

	All neonates n = 236		Eligible n = 117		Ineligible n = 119	
Demographics	n	(%)	n	(%)	n	(%)
Sex						
Female	116	(49.2)	54	(46.2)	62	(52.1)
Ethnicity						
New Zealand European	160	(67.8)	73	(62.4)	87	(73.1)
Māori	34	(14.4)	24	(20.5)	10	(8.4)
Cook Island Māori	4	(1.7)	2	(1.7)	2	(1.7)
Tongan	4	(1.7)	1	(0.9)	3	(2.5)
Indian	2	(0.9)	1	(0.9)	1	(0.8)
Chinese	1	(0.4)	1	(0.9)	0	(0.0)
Samoan	1	(0.4)	1	(0.9)	0	(0.0)
Niuean	0	(0.0)	0	(0.0)	0	(0.0)
Other*	28	(11.9)	13	(11.1)	15	(12.6)
Birth						
GA, weeks, mean (sd)	34.3	(± 0.3)	33.0	(± 0.4)	35.7	(± 0.4)
Birth weight, g, mean (sd)	2,327	(± 63.4)	1,981	(± 77.7)	2,667	(± 89.9)
Delivery type †						
Caesarian section	130	(55.1)	67	(57.3)	63	(52.9)
Vaginal delivery tools						
None	92	(87.6)	43	(87.8)	49	(87.5)
Forceps	7	(3.0)	5	(4.3)	2	(1.7)
Ventouse	9	(3.8)	2	(1.7)	7	(5.9)
Multiple births	52	(22.0)	31	(26.5)	21	(17.7)
Born in Dunedin Hospital	209	(88.6)	104	(88.6)	105	(88.2)
In the NICU						
Length of stay, days, mean (sd)	22.6	(± 1.4)	30.5	(± 2.0)	14.7	(± 1.9)
Vital status at discharge						
Deceased	4	(1.7)	2	(1.7)	2	(1.7)

NICU - neonatal intensive care unit; sd – standard deviation; GA – gestational age.
*Other ethnicities included: European not further defined (n=11), Asian not further defined (n=2), Eurasian not further defined (n=2), Southeast Asian (n=2), Fijian Indian (n=1), Middle Eastern not further defined (n=1), North American/German (n=1), Pacific not further defined (n=1), Papa New Guinean (n=1), South African (n=1), Swiss European (n=1), Syrian (n=1), Tokelauan (n=1), UK European (n=1), and Venezuelan (n=1).
† Delivery type missing for one neonate.

5.2.2 Eligible colonised and non-colonised neonates

Table 3 shows the baseline characteristics of the 117 eligible, 42 colonised, and 75 non-colonised neonates. Among the colonised neonates, 14 (33.3%) were female. Among the non-colonised neonates, 40 (53.3%) were female. Among the colonised neonates, 24 (57.1%) were New Zealand European compared to 49 (65.3%) among the non-colonised neonates.

The mean (standard deviation) gestational age at birth was 29.6 (± 0.5) weeks for colonised neonates and 34.8 (± 0.4) weeks for non-colonised neonates. The mean (standard deviation) birthweight was 1,348 (± 82.2) g for colonised neonates and 2,335 (± 89.1) g for non-colonised neonates. The mean (standard deviation) length of stay was 42.9 (± 3.5) days for colonised neonates and 23.6 (± 2.0) days for non-colonised neonates. Birth was by caesarean section for 30 (71.4%) colonised neonates and 37 (59.3%) non-colonised neonates. Fifteen (35.7%) colonised neonates were born as a part of a multiple birth compared to 15 (21.3%) non-colonised neonates.

Table 3: Baseline characteristics of the eligible neonates by colonised and non-colonised status, Dunedin Hospital NICU, September 2013 through March 2015

	Eligible n =117		Colonised n = 42		Non-colonised n = 75	
Demographics	n	(%)	n	(%)	n	(%)
Sex						
Female	54	(46.2)	14	(33.3)	40	(53.3)
Ethnicity						
New Zealand European	73	(62.4)	24	(57.1)	49	(65.3)
Māori	24	(20.5)	9	(21.4)	15	(20.0)
Cook Island Māori	2	(1.7)	2	(4.8)	0	(0.0)
Chinese	1	(0.9)	1	(2.4)	0	(0.0)
Indian	1	(0.9)	0	(0.0)	1	(1.3)
Samoan	1	(0.9)	0	(0.0)	1	(1.3)
Tongan	1	(0.9)	0	(0.0)	1	(1.3)
Niuean	0	(0.0)	0	(0.0)	0	(0.0)
Other*	13	(11.1)	6	(13.7)	6	(8.0)
Birth						
GA, weeks, mean (sd)	33.0	(± 0.4)	29.6	(± 0.5)	34.8	(± 0.4)
Birth weight, g, mean (sd)	1,981	(± 77.7)	1,348	(± 82.2)	2,335	(± 89.1)
Delivery type						
Caesarian section	67	(57.3)	30	(71.4)	37	(49.3)
Vaginal delivery tools						
None	43	(87.8)	11	(26.2)	32	(43.2)
Forceps	5	(4.3)	1	(2.4)	4	(5.3)
Ventouse	2	(1.7)	0	(0.0)	2	(2.7)
Multiple births	31	(26.5)	15	(35.7)	15	(21.3)
Born in Dunedin Hospital	104	(88.6)	38	(90.5)	66	(88.0)
In the NICU						
Length of stay, days, mean (sd)	30.5	(± 2.0)	42.9	(±3.5)	23.6	(± 2.0)
Vital status at discharge						
Deceased	2	(1.7)	1	(2.4)	1	(1.4)

NICU - neonatal intensive care unit; sd – standard deviation; GA – gestational age.
* Other ethnicities included: European not further defined (n=6), Asian not further defined (n=1), North American/German (n=1), Pacific not further defined (n=1), Syrian (n=1), Tokelauan (n=1), UK European (n=1), and Venezuelan (n=1).

5.2.3 Eligible neonates with or without weekly data

Weekly data were available for eligible neonates among whom we could access medical records. Of the 64 neonates with available weekly data, 26 (40.6%) were colonised neonates, and 38 (59.4%) were non-colonised neonates. Of the 53 neonates without available weekly data, 16 (30.2%) were colonised neonates, and 37 (69.8%) were non-colonised neonates. Table 4 shows the baseline characteristics of eligible neonates with or without weekly data. Among the neonates with available weekly data, 28 (43.8%) were female. Among the neonates without available weekly data, 26 (49.1%) were female. Seventeen (26.6%) neonates with available weekly data were Māori, compared to 7 (13.2%) neonates without available weekly data. Fifteen (23.4%) neonates with available data were born as part of a multiple birth compared to 16 (30.2%) among the neonates without available data. The mean (standard deviation) length of stay was 37.4 (±3.2) days for neonates with weekly data and 22.2 (±1.5) days for those without weekly data.

Table 4: Baseline characteristics of the eligible neonates by availability, Dunedin Hospital NICU, September 2013 through March 2015

	Available weekly data n= 64		Unavailable weekly data n = 53	
Demographics	n	(%)	n	(%)
Sex				
Female	28	(43.8)	26	(49.1)
Ethnicity				
New Zealand European	39	(60.9)	35	(66.1)
Māori	17	(26.6)	7	(13.2)
Cook Island Māori	1	(1.6)	1	(1.9)
Chinese	1	(1.6)	0	(0.0)
Indian	0	(0.0)	1	(1.9)
Samoan	1	(1.6)	0	(0.0)
Tongan	1	(1.6)	0	(0.0)
Niuean	0	(0.0)	0	(0.0)
Other*	4	(6.3)	9	(17.0)
Birth				
GA, weeks, mean (sd)	32.7	(±0.5)	33.2	(±0.6)
Birthweight, g, mean (sd)	1,906	(±108.3)	2,070	(±110.8)
Delivery type				
Caesarian section	36	(56.3)	31	(58.5)
Vaginal delivery tools †				
None	24	(85.7)	20	(90.9)
Forceps	3	(4.7)	2	(3.8)
Ventouse	2	(3.1)	0	(0.0)
Multiple births	15	(23.4)	16	(30.2)
Born in Dunedin Hospital	56	(87.5)	48	(90.6)
In the NICU				
Length of stay, days, mean (sd)	37.4	(±3.2)	22.2	(±1.5)
Vital status at discharge				
Deceased	2	(3.1)	0	(0.0)

NICU - neonatal intensive care unit; sd – standard deviation; GA – gestational age.
* Other ethnicities included: European not further defined (n=6), Asian not further defined (n=1), North American/German (n=1), Pacific not further defined (n=1), Syrian (n=1), Tokelauan (n=1), UK European (n=1), and Venezuelan (n=1).
† One neonate with available weekly data had both forceps and ventouse used in their vaginal delivery.

5.3 Nested case-control study

Of the 64 neonates with available weekly data, 26 (40.6%) colonised neonates were included in the nested case-control study as cases, and 38 (59.4%) non-colonised neonates were potential controls. Each of the cases were matched to controls based on the matching criteria (section 4.17.3). This gave a total of 203 weeks of control data, contributed by 38 individual neonates, median number of controls per case of 7.8, range (1, 21). Among the 38 individual neonates that contributed control data, 24 (63.2%) were non-colonised neonates, and 14 (36.8%) were colonised neonates who also contributed case data. Fourteen (36.8%) non-colonised neonates did not contribute weekly data, as their control weeks did not meet matching criteria with cases. Of the 203 weeks of control data, 120 (59.1%) weeks were contributed by colonised neonates, and 83 (40.9%) weeks were contributed by non-colonised neonates. Of the 14 colonised neonates that contributed case and control data, all 14 had control data matched to their own case data.

5.4 Nested case-control unadjusted analysis

Baseline characteristics of colonised and non-colonised neonates that contributed weekly data

Table 5 shows the baseline characteristics for the 26 colonised neonates and 24 non-colonised neonates who contributed weekly data to the matched case-control analysis. Of colonised neonates, 5 (19.2%) were female whereas 12 (50.0%) non-colonised neonates were female. The mean (standard deviation) gestational age at birth for colonised neonates was 29.7 (± 0.6) weeks and for non-colonised neonates was 34.4 (± 0.7) weeks. The mean (standard deviation) birthweight for colonised neonates was 1,374 (±108.9) g, and for non-colonised neonates was 2,198 (±155.4) g. Caesarean sections were the mode of delivery for 20 (76.9%) colonised neonates and 11 (45.8%) non-colonised neonates. The mean (standard deviation) length of stay was 50.5 (± 4.9) among colonised neonates and 30.2 (± 5.3) days among non-colonised neonates.

Table 5: Baseline characteristics of colonised and non-colonised neonates that contributed data to the matched case-control analysis, Dunedin Hospital NICU, September 2013 through March 2015

	Colonised neonates n = 26		Non-colonised neonates n = 24	
Demographics	n	(%)	n	(%)
Sex				
Female	5	(19.2)	12	(50.0)
Ethnicity*				
Non-Māori	20	(76.9)	16	(66.7)
Māori	6	(23.1)	7	(29.2)
Birth				
GA, weeks, mean (sd)	29.7	(± 0.6)	34.4	(± 0.7)
≤32 weeks	18	(69.2)	5	(20.8)
>32 weeks	8	(30.8)	19	(79.2)
Birth weight, g, mean (sd)	1,374	(±108.9)	2,198	(±155.4)
≤1500g	19	(73.1)	3	(12.5)
>1500g	7	(26.9)	21	(87.5)
Delivery type				
Caesarian section	20	(76.9)	11	(45.8)
Vaginal delivery tools †				
None	6	(23.1)	11	(45.8)
Forceps	0	(0.0)	1	(4.2)
Ventouse	0	(0.0)	1	(4.2)
Multiple births	8	(30.8)	7	(29.2)
Born in Dunedin Hospital	25	(96.2)	21	(87.5)
In the NICU				
Length of stay, days, mean (sd)	50.5	(± 4.9)	30.2	(± 5.3)
≤40 days	11	(42.3)	19	(79.2)
>40 days	15	(57.7)	5	(20.8)
Vital status at discharge				
Deceased	1	(3.9)	0	(0.0)

NICU – neonatal intensive care unit; sd – standard deviation; GA – gestational age.
* Ethnicity missing for one non-colonised neonate.
† One neonate with available weekly data had both forceps and ventouse used in their vaginal delivery.

Figure 7 shows number of weeks in the NICU before colonised neonates had their positive swab result for *S. capitis*. Among the 26 cases, 11 (42.3%) became colonised with *S. capitis* during their first exposure week. The number of neonates that became colonised reduced per week of stay in the NICU. No neonates became cases after their fifth week in the NICU.

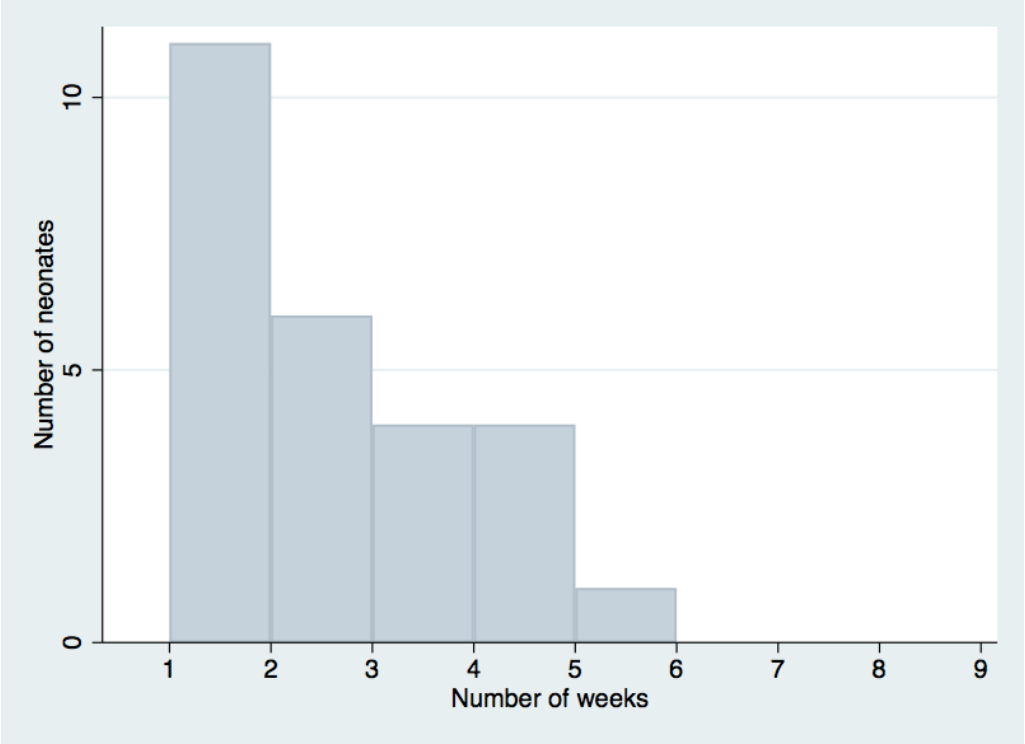

Figure 7: Number of weeks in the NICU before colonised neonates had their positive swab result for *S. capitis*

Baseline characteristics of cases and controls

Table 6 shows the baseline characteristics for the 26 cases and the 203 matched controls. The odds of being born at ≤32 weeks' gestation was 3.5 times higher among cases than among controls (OR 3.5, 95% CI 1.3-9.6, p=0.02). The odds of being born ≤1500g was 4.0 times higher among cases than among controls (OR 4.0, 95% CI 1.1-14.9, p=0.04). The odds of being born as a part of a multiple birth was 76% lower among cases than among controls (OR 0.24, 95% CI 0.08-0.67, p=0.007). The difference between cases and controls for length of stay was not statistically significant (OR 1.5, 95% CI 0.67-3.4, p=0.3).

Table 6: Baseline characteristics of cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n= 26		Controls n= 203		Unadjusted		p-value
Demographics	n	(%)	n	(%)	OR	(95% CI)	
Sex							
Female	5	(19.2)	54	(26.6)	0.68	(0.23-2.1)	0.5
Ethnicity*							
Non-Māori	20	(76.9)	157	(77.3)	1.3	(0.50-3.4)	0.6
Māori	6	(23.1)	45	(22.2)	1	(reference)	
Birth							
Gestational age							
weeks, mean (sd)	29.7	(± 0.6)	31.1	(± 0.2)	0.84	(0.75-0.94)	0.003
≤32 weeks	18	(69.2)	98	(48.3)	3.5	(1.3-9.6)	0.02
>32 weeks	8	(30.8)	105	(51.7)	1	(reference)	
Birth weight							
g, mean (sd) †	1,374	(±108.9)	1,546	(± 41.3)	0.9	(0.8-1.0)	0.1
≤1500g	19	(73.1)	103	(50.7)	4.0	(1.1-14.9)	0.04
>1500g	7	(26.9)	100	(49.3)	1	(reference)	
Delivery type							
Caesarian section	20	(76.9)	162	(79.8)	2.2	(0.41-12.4)	0.4
Vaginal delivery							
None	6	(23.1)	34	(16.8)	0.61	(0.13-3.0)	0.5
Forceps	0	(0.0)	3	(1.5)		NA	
Ventouse	0	(0.0)	4	(2.0)		NA	
Multiple births	8	(30.8)	120	(59.1)	0.24	(0.08-0.67)	0.007
Born in Dunedin Hospital	25	(96.2)	186	(91.6)	4.1	(0.60-27.8)	0.2
In the NICU							
Length of stay							
days, mean (sd)	50.5	(± 4.9)	48.2	(± 1.8)	1.0	(0.99-1.0)	0.3
≤40 days	11	(42.3)	95	(46.8)	1	(reference)	
>40 days	15	(57.7)	108	(53.2)	1.5	(0.67-3.4)	0.3
Vital status at discharge							
Deceased	1	(3.9)	0	(0.0)		NA	

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.

NA - no cases, or cell numbers less than 3; NICU – neonatal intensive care unit; sd – standard deviation.

*Ethnicity missing for one non-colonised neonate.

†Birthweight was per 100g for the univariate analysis.

5.4.1 Medications

Antimicrobial use

Antimicrobial use by cases and controls is presented in Table 7. The differences between cases and controls during the exposure week were not statistically significant for the use of any specific antimicrobials. None of the cases used fusidic acid compared to 36 (17.7%) of the controls. The OR and 95% CI could not be estimated for fusidic acid due the lack of cases.

Table 7: Unadjusted analysis of medication use in cases and controls, Dunedin Hospital NICU, September 2013 through March 2015.

Antimicrobials	Cases n= 26		Controls n= 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	(95% CI)	
Any antimicrobial	19	(73.1)	113	(55.7)	1.9	(0.54-6.6)	0.3
Antibacterials							
Amoxicillin	13	(50.0)	68	(33.3)	1.5	(0.58-3.9)	0.4
Gentamicin	8	(30.8)	35	(17.2)	1.4	(0.52-3.7)	0.5
Fusidic acid	0	(0.0)	36	(17.7)		NA	
Metronidazole	1	(3.9)	32	(15.8)	0.2	(0.03-1.9)	0.2
Amikacin	4	(15.4)	23	(11.3)	1.5	(0.41-5.3)	0.5
Chloramphenicol	1	(3.9)	9	(4.4)	1.1	(0.13-8.6)	1.0
Cefotaxime	2	(7.7)	6	(3.0)	1.9	(0.23-15.9)	0.5
Augmentin	0	(0.0)	0	(0.0)		NA	
Ceftazadime	0	(0.0)	0	(0.0)		NA	
Erythromycin	0	(0.0)	0	(0.0)		NA	
Penicillin	0	(0.0)	0	(0.0		NA	
Vancomycin	0	(0.0)	0	(0.0)		NA	
Antifungals							
Fluconazole	14	(53.9)	69	(33.8)	2.1	(0.85-5.4)	0.1
Nystatin	0	(0.0)	6	(3.0)		NA	
Clotrimazole	0	(0.0)	2	(1.0)		NA	
S. capitis resistance							
Composite *	13	(50.0)	84	(41.4)	1.2	(0.45-3.3)	0.7

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.
NA - no cases, or cell numbers less than 3; NICU – neonatal intensive care unit.
* Composite antimicrobial includes antibacterials against which *S. capitis* has resistance: amoxicillin, gentamicin, fusidic acid, metronidazole, and cefotaxime.

Antimicrobial use among colonised and non-colonised neonates that contributed weekly data

Table 8 shows the antimicrobial use among the 26 colonised neonates and 24 non-colonised neonates that contributed data to the matched case-control analysis. Among the 26 colonised neonates, 23 (88.5%) used any antimicrobial. Among the 24 non-colonised neonates, 7 (29.2%) used any antimicrobial. The composite variable of antimicrobials to which *S. capitis* is resistant includes amoxicillin, gentamicin, fusidic acid, metronidazole, and cefotaxime. Antimicrobials to which *S. capitis* is resistant were used by 20 (76.9%) colonised neonates, and 5 (20.8%) non-colonised neonates.

Table 8: Antimicrobials used by colonised and non-colonised neonates that contributed data to the matched case-control analysis, Dunedin Hospital NICU, September 2013 to March 2015

Antimicrobials	Colonised neonates n= 26		Non-colonised neonates n= 24	
	n	(%)	n	(%)
Any antimicrobial	23	(88.5)	7	(29.2)
Antibacterial				
Amoxicillin	20	(76.9)	4	(16.7)
Gentamicin	15	(57.7)	3	(12.5)
Fusidic acid	3	(11.5)	1	(4.2)
Metronidazole	5	(19.2)	0	(0.0)
Amikacin	6	(23.1)	0	(0.0)
Chloramphenicol	2	(7.7)	1	(4.2)
Cefotaxime	4	(15.4)	1	(4.2)
Antifungal				
Fluconazole	15	(57.7)	2	(8.3)
Nystatin	1	(3.8)	1	(4.2)
Clotrimazole	0	(0.0)	1	(4.2)
S. capitis resistance				
Composite *	20	(76.9)	5	(20.8)

NICU – neonatal intensive care unit.
* Composite antimicrobial includes antibacterials against which *S. capitis* has resistance: amoxicillin, gentamicin, fusidic acid, metronidazole, and cefotaxime.

Other medications

Medications other than antimicrobials used by cases and controls, and the univariate analysis are shown in Table 9. Probiotics are used to promote healthy gut microflora and prevent necrotising enterocolitis (NEC) (137). Infloran probiotics contain *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. The odds of infloran probiotic use was 5.2 times higher among cases than among controls (OR 5.2, 95% CI: 1.5–18.0, p=0.009). Caffeine was used among neonates to reduce apnoeas of prematurity, extubation failure, and patent ductus arteriosus (PDA) (138-140). The odds of caffeine use was 5.2 times higher among cases than among controls (OR 5.2, 95% CI: 1.1-24.5, p=0.04).

Vitadol C is a vitamin supplement which contains vitamin A, vitamin D, and vitamin C. The odds of Vitadol C use was 1.5 times higher among cases than among controls (OR 1.5, 95% CI: 0.15-5.4, p=0.9). The odds of oral sodium chloride (NaCl) use was 10 times higher among cases than among controls (OR 10.0, 95% CI 2.0-48.8, p=0.004). The diuretics combination of chlorthiazide and spironolactone are used to reduce the risk of development of chronic lung disease (CLD) in neonates. The odds of chlorthiazide and spironolactone use was 4.3 times higher among cases than among controls (OR 4.3, 95% CI: 1.5-13.0, p=0.009). The ORs and 95% CIs for all of the non-antimicrobial topical creams could not be calculated accurately as there were no observations for the cases.

Table 9: Unadjusted analysis of other medication use in cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n= 26		Controls n= 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	(95% CI)	
Any medication	25	(96.2)	193	(94.6)	0.76	(0.07-7.9)	0.8
Infloran probiotics*	23	(88.5)	129	(63.6)	5.2	(1.5-18.0)	0.009
Caffeine	21	(80.8)	125	(61.6)	5.2	(1.1-24.5)	0.04
Micelle E †	7	(26.9)	44	(21.7)	2.5	(0.73-8.8)	0.1
Glycerine	0	(0.0)	43	(21.2)		NA	
Gaviscon infants‡	1	(3.9)	4	(2.0)	1.3	(0.15-10.4)	0.8
Dopamine	0	(0.0)	4	(2.0)		NA	
Insulin	0	(0.0)	4	(2.0)		NA	
Ibuprofen	1	(3.9)	0	(0.0)		NA	
Supplements							
Vitadol C§	12	(46.2)	141	(69.5)	0.47	(0.15-1.4)	0.2

	Cases n= 26		Controls n= 203		Unadjusted		p-value
Supplements	n	(%)	n	(%)	OR	(95% CI)	
Phosphate	2	(7.7)	21	(10.3)	1.2	(0.24-5.4)	0.9
Iron	1	(3.9)	19	(9.4)	1.5	(0.05-48.0)	0.8
Oral NaCl	4	(15.4)	7	(3.5)	10.0	(2.0-48.8)	0.004
KCl	1	(3.9)	10	(4.9)	0.38	(0.04-3.5)	0.4
Calcium gluconate	0	(0.0)	5	(2.5)		NA	
Duocal paste ‖	0	(0.0)	3	(1.5)		NA	
Pain relief							
Sucrose ¶	3	(11.5)	8	(3.9)	1.9	(0.44-7.7)	0.4
Morphine	0	(0.0)	2	(1.0)		NA	
Paracetamol	1	(3.9)	0	(0.0)		NA	
Topical creams							
Sudocrem ◊	0	(0.0)	4	(2.0)		NA	
Bepanthen *f*	0	(0.0)	4	(2.0)		NA	
Zinc oxide	0	(0.0)	2	(1.0)		NA	
Diuretics							
Chlorthiazide and spironolactone	3	(11.5)	7	(3.5)	4.3	(1.5-13.0)	0.009
Frusemide	1	(3.9)	3	(1.5)	6.1	(0.83-44.9)	0.08
Amiloride	0	(0.0)	3	(1.5)		NA	
Injectables							
TPN	16	(61.5)	91	(44.8)	1.9	(0.46-7.6)	0.4
Lipids	16	(61.5)	89	(43.8)	2.0	(0.49-8.4)	0.3
Dextrose	12	(46.2)	67	(33.0)	1.0	(0.45-2.4)	0.9
IV NaCl	9	(34.6)	39	(19.2)	1.6	(0.59-4.4)	0.4
Glucose	3	(11.5)	17	(8.4)	0.96	(0.26-3.5)	1.0
Heparinised saline	3	(11.5)	10	(4.9)	1.2	(0.26-5.9)	0.8
Iopromide	0	(0.0)	4	(2.0)		NA	
Suxamethonium, atropine and fentanyl	0	(0.0)	5	(2.5)		NA	

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.

NA - no cases, or cell numbers less than 3; NICU – neonatal intensive care unit; TPN – total parental nutrition.

* Infloran probiotics contains *Bifidobacterium bifidum* and *Lactobacillus acidophilus*.

† Micelle E is a vitamin E formulation given for prevention of retinopathy of prematurity.

‡ Gaviscon infants contains sodium alginate and sodium bicarbonate.

§ Vitadol C contains vitamin A, vitamin D and vitamin C.

‖ Duocal paste a nutritional supplement that contains protein and carbohydrate

¶ Oral sucrose is given as a pain relief for minor procedures.

◊ Sudocrem contains zinc oxide and benzyl alcohol.

f Bepanthen contains dexpanthenol and lanolin.

5.4.2 Medical history

The unadjusted analysis of medical history of the cases and controls is shown in Table 10. Temperature instability is defined by a neonate's requirement for external temperature control using incubator controls, or an adjustment of their amount of clothing. The odds of temperature instability was 2.7 times higher among cases than among controls (OR 2.7, 95% CI 1.0-6.8, p=0.04). The odds of skin inflammation was 72% lower among cases than among controls (OR 0.28, 95% CI 0.1-0.7, p=0.009). The difference between cases and controls for skin inflammation was not statistically significant for any specific body sites. The odds of cardiac abnormalities was 2.6 times higher among cases than among controls (OR 2.6, 95% CI: 1.1-6.0, p=0.03). The odds of PDA was 4.2 times higher among cases than among controls (OR 4.2, 95% CI: 1.6-10.7, p=0.003). The differences between cases and controls were not statistically significant for any other cardiac abnormalities in the exposure week. The odds of CLD was 8.5 times higher among cases than among controls (OR 8.5, 95% CI: 0.6-10.5, p=0.009).

Table 10: Unadjusted analysis of the medical history of cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n = 26		Controls n = 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	95% CI	
Feed intolerance	6	(23.1)	59	(29.1)	0.5	(0.13-1.7)	0.2
Gastric aspirate	6	(23.1)	58	(28.6)	0.5	(0.14-2.1)	0.4
Temperature instability*	13	(50.0)	49	(24.1)	2.7	(1.0-6.8)	0.04
Skin injury	5	(19.2)	41	(20.2)	0.95	(0.32-2.9)	0.9
Sepsis workup	5	(19.2)	27	(13.3)	1.9	(0.71-5.3)	0.2
Intracranial haemorrhage	0	(0.0)	14	(6.9)		NA	
Hypoglycaemia	0	(0.0)	12	(5.9)		NA	
Renal impairment	0	(0.0)	12	(5.9)		NA	
Flaky skin	1	(3.9)	7	(3.5)	0.8	(0.07-8.1)	0.8
Sepsis diagnosis	2	(7.7)	3	(1.5)	8.6	(0.86-86.2)	0.07
Neonatal encephalopathy	0	(0.0)	4	(2.0)		NA	
Umbilical flare	1	(3.9)	2	(1.0)		NA	
Retinopathy of prematurity	0	(0.0)	0	(0.0)		NA	
Area of inflamed skin							
Yes, total	8	(30.8)	119	(58.6)	0.28	(0.11-0.73)	0.009
Axilla	2	(7.7)	48	(23.7)	0.28	(0.06-1.4)	0.1
Eye	3	(11.5)	30	(14.8)	1.2	(0.33-4.3)	0.8
Buttocks	2	(7.7)	27	(13.3)	0.38	(0.08-1.9)	0.2
Intravenous line	1	(3.9)	23	(11.3)	0.3	(0.04-2.5)	0.3

	Cases n = 26		Controls n = 203		Unadjusted		p-value
Area of inflamed skin	n	(%)	n	(%)	OR	95% CI	
Ear	0	(0.0)	7	(3.5)		NA	
Full body	0	(0.0)	5	(2.5)		NA	
Groin	1	(3.9)	3	(1.5)	1.7	(0.26-11.8)	0.6
Oral thrush	0	(0.0)	4	(2.0)		NA	
Neck	1	(3.9)	2	(1.0)		NA	
Gastrointestinal disease							
Yes, total	0	(0.0)	18	(8.9)		NA	
Necrotising enterocolitis	0	(0.0)	14	(6.9)		NA	
Bowel ischaemia	0	(0.0)	4	(2.0)		NA	
Cardiac abnormalities							
Yes, total	13	(50.0)	55	(27.1)	2.6	(1.1-6.0)	0.03
Patent ductus arteriosus	12	(46.2)	31	(15.3)	4.2	(1.6-10.7)	0.003
Murmur	5	(19.2)	37	(18.2)	0.94	(0.35-2.5)	0.9
Septal defects	1	(3.9)	14	(6.9)	0.5	(0.05-5.5)	0.6
Ventricular impairment	1	(3.9)	8	(3.9)	1.1	(0.13-9.9)	0.9
Artery impairment	0	(0.0)	2	(1.0)		NA	
Valve impairment	1	(3.9)	0	(0.0)		NA	
Tachycardia	1	(3.9)	0	(0.0)		NA	
Pulmonary disease							
Yes, total	12	(46.2)	96	(47.3)	1.7	(0.44-6.7)	0.2
RDS	12	(46.2)	88	(42.4)	2.5	(0.61-10.5)	0.2
Chronic lung disease	4	(15.4)	8	(3.9)	8.5	(1.6-42.9)	0.009
Pulmonary hypoplasia	1	(3.9)	3	(1.5)	5.1	(0.79-33.5)	0.09
Emphysema	1	(3.9)	0	(0.0)		NA	
Pneumothorax	1	(3.9)	0	(0.0)		NA	
Pulmonary haemorrhage	0	(0.0)	1	(0.5)		NA	

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.

NA - no cases, or cell numbers less than 3; RDS – respiratory distress syndrome.

* Temperature instability is defined by a neonate's requirement for external temperature control using incubator controls, or an adjustment of their amount of clothing.

Inflamed skin of colonised and non-colonised neonates that contributed weekly data

Table 11 shows the areas of inflamed skin for colonised and non-colonised neonates who contributed weekly data to the matched case-control analysis. Among the 26 cases, 12 (46.2%) had an area of inflamed skin. Among the 24 controls, 16 (66.7%) had an area of inflamed skin. Five (19.2%) colonised neonates and one (4.2%) non-colonised neonate had an inflamed axilla. Four (15.4%) cases and one (4.2%) control had inflamed skin at the site of an intravenous line.

Table 11: Areas of inflamed skin identified among colonised and non-colonised neonates, Dunedin Hospital NICU, September 2013 through March 2015

Inflamed skin	Colonised neonates n= 26		Non-colonised neonates n= 24	
	n	(%)	n	(%)
Any area	12	(46.2)	16	(66.7)
Axilla	5	(19.2)	1	(4.2)
Eye	3	(11.5)	3	(12.5)
Buttocks	3	(11.5)	11	(45.8)
IV line	4	(15.4)	1	(4.2)
Ear	1	(3.8)	0	(0.0)
Full body	1	(3.8)	2	(8.3)
Groin	1	(3.8)	2	(8.3)
Oral thrush	0	(0.0)	2	(8.3)
Neck	1	(3.8)	1	(4.2)

NICU – neonatal intensive care unit; IV – intravenous.

5.4.3 Procedures and devices

The descriptive data for cases and controls, along with the unadjusted analysis for procedures and devices are shown in Table 12. The differences between cases and controls was not statistically significant for the use of peripherally inserted central catheters, umbilical artery catheters, or umbilical venous catheters. The odds of requiring invasive mechanical ventilation was 5.3 times higher among cases than among controls (OR 5.3, 95% CI: 1.6-17.7, p=0.007). Compared with no phototherapy, the odds of phototherapy for 3-6 days was 3.3 times higher among cases than among controls (OR 3.3, 95% CI 1.1-9.6, p=0.03).

Table 12: Unadjusted analysis of the procedures and devices required by cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n = 26		Controls n = 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	(95% CI)	
Nasogastric tube	16	(61.5)	142	(70.0)	0.64	(0.22-2.1)	0.5
Peripheral IV cannula	16	(61.5)	128	(63.1)	0.5	(0.14-1.7)	0.3
Orogastric tube	16	(61.5)	112	(55.2)	1.2	(0.27-4.8)	0.8
Nasal CPAP	14	(53.9)	117	(57.6)	1.0	(0.33-3.0)	1.0
PICC	16	(61.5)	77	(37.9)	2.2	(0.69-7.1)	0.2
UVC	7	(26.9)	23	(11.3)	1.5	(0.44-4.9)	0.5
UAC	3	(11.5)	10	(4.9)	1.2	(0.26-5.9)	0.8
Invasive mechanical ventilation	5	(19.2)	6	(3.0)	5.3	(1.6-17.7)	0.007
Endotracheal intubation	2	(7.7)	4	(2.0)	3.0	(0.76-11.5)	0.1
Nasal cannula	0	(0.0)	6	(3.0)		NA	
ROP screen	0	(0.0)	4	(2.0)		NA	
Blood tests							
Number, mean (sd)	12.2	(± 2.3)	8.5	(±0.6)	1.0	(0.98-1.1)	0.3
Phototherapy							
None	16	(61.5)	135	(66.5)	1	(reference)	
1-2 days	5	(19.2)	58	(28.6)	0.48	(0.19-1.2)	0.1
3-6 days	5	(19.2)	10	(4.9)	3.3	(1.1-9.6)	0.03
RBC transfusion							
Number, mean (sd)	1.2	(± 0.2)	1.6	(±0.2)		NA	
Apnoeas *							
Number, mean (sd)	8.8	(± 1.8)	10.7	(± 0.7)	1.0	(0.90-1.1)	1.0
Type of stimulation							
Gentle	15	(57.7)	111	(54.7)	1.5	(0.63-3.4)	0.4
Moderate	3	(11.5)	42	(20.7)	0.54	(0.14-2.0)	0.4
Vigorous	0	(0.0)	8	(3.9)		NA	
Funnel/facial O_2	11	(42.3)	105	(51.7)	0.92	(0.42-2.0)	0.8

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.

NA - no cases, or cell numbers less than 3; IV – intravenous; CPAP – continuous positive airway pressure; PICC – peripherally inserted central catheter; UVC – umbilical vein catheter; UAC – umbilical artery catheter; ROP - retinopathy of prematurity; sd – standard deviation; O_2 – oxygen.

*All apnoeas required stimulation, some neonates required more than one type of stimulation per apnoea.

Procedures performed outside the NICU or by allied health professionals

The data regarding procedures performed outside of the NICU or by allied health professionals are shown in Table 13. The odds of having a head ultrasound scan (HUSS) was 2.5 times higher among cases than among controls (OR 2.5, 95% CI 1.1-5.8, p=0.03). Procedures that were only performed among either cases or controls did not produce interpretable ORs or 95% CIs.

Table 13: Unadjusted analysis of procedures performed outside the NICU or by allied health professionals required by cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n = 26		Controls n = 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	(95% CI)	
Radiography	12	(46.2)	89	(43.8)	0.86	(0.32-2.3)	0.8
Head ultrasound scan	13	(50.0)	48	(23.7)	2.5	(1.1-5.8)	0.03
Echocardiogram	4	(15.4)	26	(12.8)	0.95	(0.29-3.1)	0.9
Abdominal ultrasound	1	(3.9)	19	(9.4)	0.14	(0.01-1.7)	0.1
Hearing screen	0	(0.0)	9	(4.4)		NA	
Eye check	0	(0.0)	8	(3.9)		NA	
Lumbar puncture	0	(0.0)	2	(1.0)		NA	
Electroencephalogram	0	(0.0)	1	(0.5)		NA	
Spinal ultrasound scan	1	(3.9)	0	(0.0)		NA	

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.
NA - no cases, or cell numbers less than 3; NICU – neonatal intensive care unit.

5.4.4 Enteral feeds

Descriptive information and unadjusted odds ratios comparing enteral feeds among cases and controls is shown in Table 14. Enteral feeds for neonates in the Dunedin Hospital NICU included breast milk, expressed breast milk, donor breast milk, or formula. The odds of being fed with formula was 66% lower among cases that among controls (OR 0.34, 95% CI 0.13-0.88, p=0.03). Neonates were fed via a nasogastric tube, orogastic tube, breast, bottle, syringe, cup, or finger. Neonates that are unable to use regular teats due to a cleft palate use Haberman teats. No case, and three (1.5%) controls used Haberman teats to assist with bottle feeds. The difference between cases and controls was not statistically significant for any of the modes of feeding.

Table 14: Unadjusted analysis of enteral feedings method by cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n= 26		Controls n= 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	(95% CI)	
Number of feeds per week, mean (sd)	28.9	(± 2.8)	28.4	(± 1.0)	1.0	(0.99 – 1.1)	0.2
Type of food *							
Expressed breast milk	23	(88.5)	165	(81.3)	1.7	(0.50 – 5.6)	0.4
Breast milk	6	(23.1)	48	(23.7)	1.1	(0.37 – 3.2)	0.9
Donor breast milk	0	(0.0)	0	(0.0)		NA	
Human milk fortifier	6	(23.1)	55	(27.1)	1.6	(0.50-5.3)	0.4
Formula	6	(23.1)	100	(49.3)	0.34	(0.13 – 0.88)	0.03
Mode of feeding							
NGT	13	(50.0)	123	(60.6)	0.61	(0.22 – 1.7)	0.3
OGT	16	(61.5)	113	(55.7)	1.5	(0.27 – 8.0)	0.7
Breast feeds	6	(23.1)	49	(24.1)	1.1	(0.37 – 3.1)	0.9
Bottle	2	(7.7)	31	(15.3)	0.43	(0.09 – 2.1)	0.3
Syringe	3	(11.5)	36	(17.7)	0.40	(0.11 – 1.4)	0.1
Cup	1	(3.9)	18	(8.9)	0.30	(0.03 – 3.3)	0.3
Finger	0	(0.0)	6	(3.0)		NA	
Haberman teat	0	(0.0)	3	(1.5)		NA	

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.

NA - no cases, or cell numbers less than 3; sd – standard deviation; NGT – nasogastric tube; OGT – orogastric tube

* Neonates could have more than one type of enteral feed, and none had no enteral feeds over 7 days.

5.4.5 Weight during the exposure week

The number of times the neonates were weighed, the average weight over the exposure week and the unadjusted odds ratios comparing cases and controls are shown in Table 15. There was no statistically significant difference between cases and controls for either the number of times the neonates were weighed during the exposure week, or for their average weight.

Table 15: Unadjusted analysis of the weight cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n = 26		Controls n = 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	(95% CI)	
Times weighed							
0	1	(3.9)	0	(0.0)		NA	
1	2	(7.7)	12	(5.9)	1	(reference)	
2	9	(34.6)	71	(35.0)	1.2	(0.2-7.0)	0.8
3	11	(42.3)	97	(47.8)	0.86	(0.17-4.3)	0.9
4	3	(11.5)	23	(11.3)	1.4	(0.17-11.1)	0.8
Average weight							
g, mean (sd) *	1,610	(±140.5)	1,826	(± 48.8)	0.97	(0.90-1.0)	0.4

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.
NA - no cases, or cell numbers less than 3; NICU – neonatal intensive care unit; sd – standard deviation.
*Average weight was per 100g for the univariate analysis.

5.4.6 Type of bed, bed spaces, and rooms

The unadjusted analysis of the type of bed, bed spaces, and rooms that were occupied by cases and controls is shown in Table 16. The difference between cases and controls was not statistically significant for the type of bed they occupied, nor if they changed from incubator to cot.

The Dunedin Hospital NICU has 16 rooms and 26 bed spaces for neonates (Figure 3). The rooms occupied most often are shown in Figures 8 and 9. The differences between cases and controls were not statistically significant for any of the bed spaces or rooms.

Table 16: Bed spaces occupied during cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n= 26		Controls n= 203		Unadjusted		p-value
	n	(%)	n	(%)	OR	(95% CI)	
Type of bed							
Incubator	21	(80.8)	162	(79.8)	1.2	(0.34-4.4)	0.8
Cot	8	(30.8)	86	(42.4)	0.48	(0.14-1.6)	0.2
Change, incubator to cot	4	(15.4)	61	(30.1)	0.30	(0.07-1.3)	0.1
Bed space *							
2	0	(0.0)	0	(0.0)		NA	
3	0	(0.0)	4	(2.0)		NA	
4	0	(0.0)	0	(0.0)		NA	
5a	2	(7.7)	12	(5.9)	1.8	(0.28-10.9)	0.5
5b	0	(0.0)	8	(3.9)		NA	
5c	0	(0.0)	8	(3.9)		NA	
5d	0	(0.0)	3	(1.5)		NA	
6a	3	(11.5)	13	(6.4)	0.72	(0.20-2.5)	0.6
6b	2	(7.7)	0	(0.0)		NA	
7a	0	(0.0)	13	(6.4)		NA	
7b	2	(7.7)	24	(11.8)	1.0	(0.24-4.4)	1.0
7c	4	(15.4)	24	(11.8)	1.7	(0.56-5.2)	0.4
7d	2	(7.7)	16	(7.9)	0.76	(0.14-4.2)	0.8
8	0	(0.0)	2	(1.0)		NA	
9	2	(7.7)	15	(7.4)	0.79	(0.17-3.6)	0.8
10	1	(3.9)	13	(6.4)	0.51	(0.05-5.3)	0.6
11a	4	(15.4)	44	(21.2)	0.95	(0.31-2.9)	1.0
11b	5	(19.2)	30	(14.8)	1.4	(0.49-3.8)	0.5
11c	2	(7.7)	14	(6.9)	1.6	(0.34-7.6)	0.5
11d	4	(15.4)	11	(5.4)	1.7	(0.32-8.6)	0.5
Bed space changes							
0	20	(76.9)	141	(69.5)	1	(reference)	
1	4	(15.4)	46	(22.7)	0.41	(0.12-1.4)	0.2
2	2	(7.7)	16	(7.9)	0.68	(0.12-3.8)	0.7
Rooms							
5	2	(7.7)	29	(14.3)	0.45	(0.07-2.7)	0.4
6	5	(19.2)	14	(6.9)	1.2	(0.41-3.6)	0.7
7	7	(26.9)	78	(38.4)	0.58	(0.20-1.7)	0.3
11	15	(57.7)	94	(46.3)	1.6	(0.74-3.7)	0.2

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=203) are all the controls from the matched case-control sets.

NA - no cases, or cell numbers less than 3, NICU – neonatal intensive care unit.

* All bed spaces recorded were in the new Dunedin Hospital NICU after the relocation 10 December 2013.

5.4.7 Movement of cases between bed spaces

This section investigates the bed spaces occupied by the cases. Figure 8 shows the bed spaces cases occupied, and the change of bed spaces for individual cases during the exposure week. Figure 8 also shows the incidence rate of *S. capitis* positive neonates per study neonate week per room. The incidence rates ranged from 0.07-0.4, with the lowest in room 5 and the highest in room 6. During the 83 week retrospective study, there were 10 weeks from 9 December 2013 (study week 13) to 10 February 2013 (study week 22) with at least one new case per week. Room 11 had the most consecutive weekly cases. In room 11, a 4 different neonates became a case each week from the 9December 2013 (study week 13) through 30 December 2013 (study week 16).

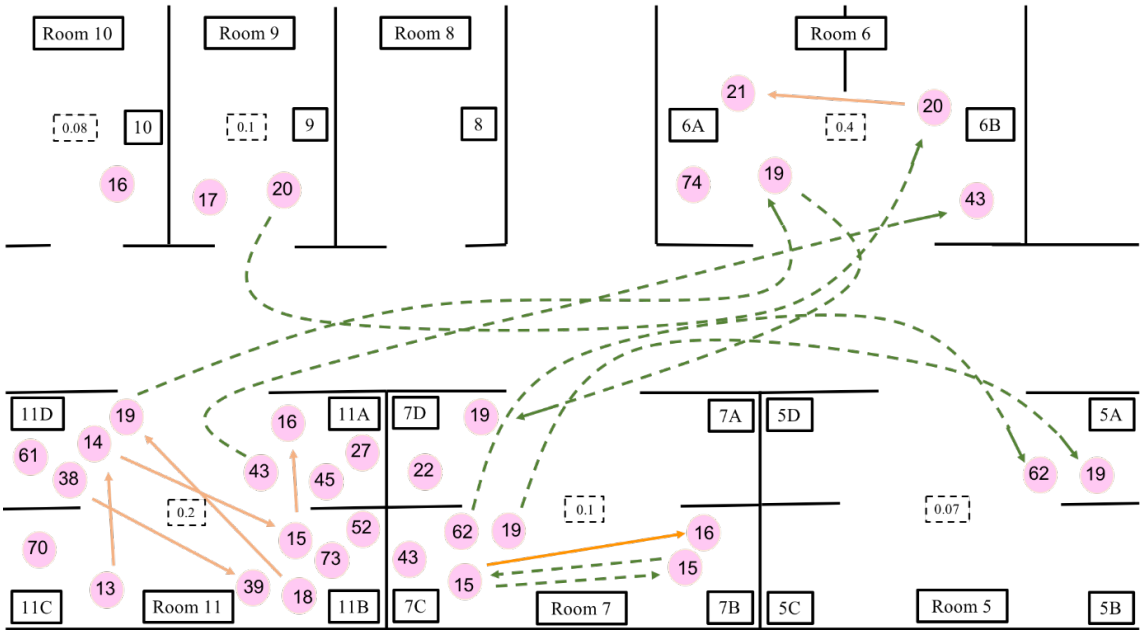

Figure 8: Diagram of the bed spaces the cases occupied during the exposure week, Dunedin Hospital NICU, 9 September 2013 (week 0) through 31 March 2015 (week 83)

- Cases — 16 - The study week number that cases had their exposure week

- Bed space movement of a single case during their exposure week

- Cases whose exposure weeks occur in consecutive weeks in the same room

0.3 - Incidence rate of the number of *S. capitis* positive neonates per study neonate week per room. Calculated as the number of cases over the number of controls per room throughout the study period

Among the neonates born as a part of a multiple birth, all were twins. Among the neonates with a twin that became a case, all became a case within one week of their twin. Twin cases in our study and the bed spaces they occupied are shown in Figure 9. Three of the four sets of twins shared the same room during their exposure weeks. One set of twins were both in bed space 7b, in a twin cot, during week 15 when the first twin became a case. The next week the second twin became a case.

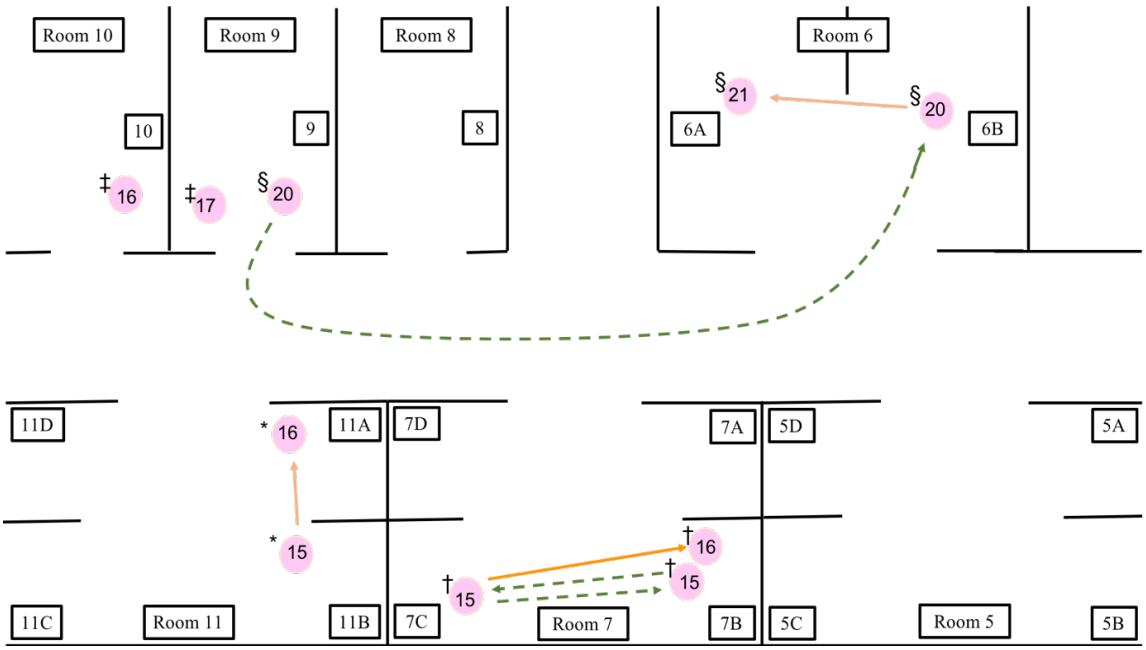

Figure 9: Diagram of the bed spaces the twins that became cases occupied during the exposure week, Dunedin Hospital NICU, September 2013 through

- Cases 16 - The study week number that cases had their exposure week

- Bed space movement of a single case during their exposure week

- Cases whose exposure weeks occur in consecutive weeks in the same room

* Twin set one, † Twin set two, ‡ Twin set three, § Twin set four.

5.5 Nested case-control adjusted analysis

The analyses adjusted for GA, and GA and length of stay for all variables with ten or more observations are shown in Table 17. After adjustment for both GA and length of stay, the odds of being born as a part of a multiple birth was 86% lower among cases than among controls (OR 0.14, 95% CI 0.04-0.40, p=<0.001). After adjusting for GA the odds of metronidazole use was 92% lower among cases than among controls (OR 0.08, 95% CI 0.01 – 0.90, p=0.04). However, after adjusting for length of stay and GA the difference between cases and controls for metronidazole use was no longer statistically significant (OR 0.08, 95% CI 0.01-1.1, p=0.06).

The difference between cases and controls for Infloran probiotic use was no longer statistically significant after adjusting for GA (OR 3.5, 95% CI 0.69-17.6, p=0.1). The difference between cases and controls for caffeine use was no longer statistically significant after adjusting for GA (OR 2.9, 95% CI: 0.50-7.0, p=0.2). After adjusting for GA and length of stay, the odds of oral NaCl use was 6.1 times higher among cases than among controls (OR 6.1, 95% CI 1.4-27.1, p=0.02). The difference between cases and controls for chlorthiazide and spironolactone use was no longer statistically significant after adjusting for GA (OR 2.8, 95% CI 0.85-9.1, p=0.09).

The difference between cases and controls for temperature instability was no longer statistically significant after adjusting for GA (OR 3.7, 95% CI 0.76-17.9, p=0.1). After adjusting for GA and length of stay, the odds of having an area of inflamed skin was 69% lower among cases than among controls (OR 0.31, 95% CI 0.13-0.70, p=0.005). The difference between cases and controls for specific areas of inflamed skin were not statistically significant after unadjusted analysis. However, after adjusting for GA, the odds of having an inflamed axilla was 91% lower among cases than among controls (OR 0.09, 95% CI 0.02-0.44, p=0.003). The difference between cases and controls for having an inflamed axilla remained statistically significant after adjusting for GA and length of stay (OR 0.31, 95% CI 0.13-0.70, p=0.005).

The difference between cases and controls for cardiac abnormalities was no longer statistically significant after adjusting for GA (OR 2.1, 95% CI: 0.80–5.5, p=0.1). After

adjusting for GA, the odds of having a PDA was 2.9 times higher among cases than among controls (OR 2.9, 95% CI 1.1-7.9, p=0.04). The difference between cases and controls for CLD was no longer statistically significant after adjusting for GA (OR 4.9 95% CI 0.97 – 24.3, p=0.06). However, after adjusting for both GA and length of stay the odds of having CLD was 4.8 times higher among cases than among controls (OR 4.8, 95% CI 1.1-22.3, p=0.04).

After adjusting for GA and length of stay, the odds of requiring invasive mechanical ventilation was 3.4 times higher among cases than among controls (OR 3.4, 95% CI 1.1-10.4, p=0.03). The difference between cases and controls for 3-6 days of phototherapy, compared to no phototherapy, was no longer statistically significant after adjusting for GA and length of stay (OR 2.5, 95% CI 0.71-8.4, p=0.2). The difference between cases and controls for requirement of a head ultrasound scan was also not statistically significant after adjusting for GA and length of stay (OR 1.3, 95% CI 0.33-5.3, p=0.7).

The difference between cases and controls for having enteral feeds with formula was no longer statistically significant after adjusting for GA (OR 0.50, 95% CI 0.19-1.3, p=0.2). However, after adjusting for both GA and length of stay the odds of having enteral feeds with formula was 71% lower among cases than among controls (OR 0.29, 95% CI 0.08-0.99, p=0.05).

Table 17: Gestational age and length of stay adjusted analysis of cases and controls, Dunedin Hospital neonatal intensive care unit, September 2013 through March 2015

	Unadjusted		p-value	Adjusted for gestational age		p-value	Adjusted for gestational age and length of stay		p-value
Baseline characteristics	OR	95% CI		OR	95% CI		OR	95% CI	
Female	0.68	(0.23-2.1)	0.5	0.50	(0.15-1.6)	0.2	0.48	(0.14-1.5)	0.2
Ethnicity									
Non- Māori	1.3	(0.50-3.4)	0.6	1.5	(0.50-4.6)	0.5	1.7	(0.48-6.2)	0.4
Māori	1	(reference)		1	(reference)		1	(reference)	
Delivery type									
Caesarean section	2.2	(0.41-12.4)	0.4	1.2	(0.17-8.3)	0.9	1.2	(0.16-8.5)	0.9
Multiple birth	0.24	(0.08-0.67)	0.007	0.14	(0.05-0.41)	<0.001	0.14	(0.04-0.4)	<0.001
Born in Dunedin Hospital	4.1	(0.60-27.8)	0.2	3.5	(0.54-23.0)	0.2	4.1	(0.68-24.7)	0.1
Antimicrobials									
Any antimicrobials	1.9	(0.54-6.6)	0.3	1.0	(0.20-5.7)	0.9	1.1	(0.20-5.6)	0.9
Antibacterials									
Amoxicillin	1.5	(0.58-3.9)	0.4	1.4	(0.52-3.9)	0.5	1.4	(0.51-3.9)	0.5
Gentamicin	1.4	(0.52-3.7)	0.5	1.5	(0.56-3.8)	0.4	1.6	(0.57-4.3)	0.4
Metronidazole	0.22	(0.03-1.9)	0.2	0.08	(0.01-0.90)	0.04	0.08	(0.01-1.1)	0.06
Amikacin	1.5	(0.41-5.3)	0.5	1.1	(0.23-5.3)	0.9	1.0	(0.18-5.5)	1.0
Chloramphenicol	1.1	(0.13-8.6)	1.0	2.1	(0.20-21.4)	0.5	2.0	(0.21-19.2)	0.5
Cefotaxime	1.9	(0.23-15.9)	0.5	2.2	(0.22-22.1)	0.5	2.2	(0.20-23.0)	0.5
Antifungals									
Fluconazole	2.1	(0.85-5.4)	0.1	0.37	(0.04-3.4)	0.4	0.35	(0.04-3.4)	0.4
S. capitis resistance									
Composite *	1.2	(0.45-3.3)	0.7	1.0	(0.34-3.1)	1.0	1.0	(0.34-3.2)	1.0
Other medications									
Any other medications	0.76	(0.07-7.9)	0.8	0.36	(0.03-3.7)	0.4	0.30	(0.03-3.3)	0.3
Infloran probiotics †	5.2	(1.5-18.0)	0.009	3.5	(0.69-17.6)	0.1	3.5	(0.75-16.3)	0.1
Caffeine	5.2	(1.1-24.5)	0.04	2.9	(0.50-7.0)	0.2	2.9	(0.53-15.5)	0.2
Micelle E ‡	2.5	(0.73-8.8)	0.1	1.4	(0.32-5.8)	0.7	1.3	(0.29-6.0)	0.7

	Unadjusted		p-value	Adjusted for gestational age		p-value	Adjusted for gestational age and length of stay		p-value
Other medications	OR	95% CI		OR	95% CI		OR	95% CI	
Supplements									
Vitadol C §	0.47	(0.15-1.4)	0.2	0.60	(0.19-1.9)	0.4	0.53	(0.16-1.8)	0.3
Iron	1.5	(0.05-48.0)	0.8	0.94	(0.02-37.1)	1.0	0.87	(0.02-33.4)	0.9
Supplements									
Oral NaCl	10.0	(2.0-48.8)	0.004	6.1	(1.3-29.2)	0.02	6.1	(1.4-27.1)	0.02
Pain relief									
Sucrose ‖	1.9	(0.44-7.7)	0.4	2.0	(0.58-7.2)	0.3	2.1	(0.57-8.1)	0.3
Diuretics									
Chlorthiazide + spironolactone	4.3	(1.5-13.0)	0.009	2.84	(0.94-8.6)	0.06	2.8	(0.85-9.1)	0.09
Injectables									
Total parental nutrition	1.9	(0.46-7.6)	0.4	0.69	(0.18-2.7)	0.6	0.64	(0.15-2.8)	0.6
Lipids	2.0	(0.49-8.4)	0.3	0.74	(0.18-3.1)	0.7	0.69	(0.15-3.2)	0.6
Dextrose	1.0	(0.45-2.4)	0.9	1.1	(0.47-2.5)	0.8	1.1	(0.47-2.5)	0.8
Glucose	0.96	(0.26-3.5)	1.0	0.51	(0.11-2.5)	0.4	0.48	(0.09-2.5)	0.4
IV NaCl	1.2	(0.41-3.4)	0.8	1.2	(0.37-3.4)	0.8	1.1	(0.34-3.4)	0.9
Heparinised saline	1.2	(0.26-5.9)	0.8	0.64	(0.11-3.7)	0.6	0.60	(0.10-3.6)	0.6
Medical history									
Feed intolerance	0.5	(0.13-1.7)	0.2	0.31	(0.08-1.2)	0.08	0.29	(0.07-1.1)	0.07
Gastric aspirate	0.5	(0.14-2.1)	0.4	0.36	(0.09-1.5)	0.2	0.33	(0.08-1.4)	0.1
Temperature instability ¶	2.7	(1.0-6.8)	0.04	3.0	(0.99-9.1)	0.05	3.7	(0.76-17.9)	0.1
Skin injury	0.95	(0.32-2.9)	0.9	0.75	(0.25-2.2)	0.6	0.75	(0.24-2.3)	0.6
Sepsis workup	1.9	(0.71-5.3)	0.2	1.9	(0.74-5.0)	0.2	1.9	(0.74-4.90)	0.2
Area of inflamed skin	0.28	(0.11-0.73)	0.009	0.30	(0.13-0.69)	0.004	0.31	(0.13-0.70)	0.005
Axilla	0.28	(0.06-1.4)	0.1	0.09	(0.02-0.44)	0.003	0.09	(0.02-0.44)	0.003
Eye	1.2	(0.33-4.3)	0.8	2.85	(0.51-15.8)	0.2	2.8	(0.51-15.0)	0.2
Buttocks	0.38	(0.08-1.9)	0.2	0.87	(0.15-5.0)	0.9	0.91	(0.15-5.6)	0.9
IV line	0.3	(0.04-2.5)	0.3	0.26	(0.05-1.4)	0.1	0.26	(0.05-1.4)	0.1
Cardiac abnormalities	2.6	(1.1-6.0)	0.03	2.1	(0.80-5.5)	0.1	2.1	(0.78-5.5)	0.1
Patent ductus arteriosus	4.2	(1.6-10.7)	0.003	2.8	(1.1-7.3)	0.03	2.9	(1.1-7.9)	0.04

	Unadjusted		p-value	Adjusted for gestational age		p-value	Adjusted for gestational age and length of stay		p-value
Cardiac abnormalities	OR	95% CI		OR	95% CI		OR	95% CI	
Murmur	0.94	(0.35-2.5)	0.9	0.70	(0.22-2.2)	0.5	0.71	(0.22-2.3)	0.6
Septal defects	0.5	(0.05-5.5)	0.6	0.37	(0.03-4.3)	0.4	0.35	(0.03-4.5)	0.4
Pulmonary disease	1.7	(0.44-6.7)	0.2	1.1	(0.37-3.3)	0.9	1.0	(0.33-3.3)	0.9
Respiratory distress syndrome	2.5	(0.61-10.5)	0.2	1.5	(0.47-4.8)	0.5	1.4	(0.40-5.2)	0.6
Chronic lung disease	8.5	(1.6-42.9)	0.009	4.9	(0.97-24.3)	0.06	4.8	(1.1-22.3)	0.04
Procedures and devices									
Nasogastric tube	0.64	(0.22-2.1)	0.5	1.8	(0.33-10.2)	0.5	1.9	(0.30-11.21)	0.5
Peripheral IV	0.5	(0.14-1.7)	0.3	0.43	(0.12-1.5)	0.2	0.40	(0.09-1.68)	0.2
Orogastric tube	1.24	(0.27-4.8)	0.8	0.26	(0.05-1.4)	0.1	0.27	(0.05-1.36)	0.1
Nasal CPAP	1.0	(0.33-3.0)	1.0	0.26	(0.06-1.2)	0.09	0.23	(0.05-1.10)	0.07
PICC	2.2	(0.69-7.1)	0.2	0.53	(0.05-5.4)	0.6	0.54	(0.05-6.05)	0.6
Umbilical vein catheter	1.5	(0.44-4.9)	0.5	0.91	(0.29-2.9)	0.9	0.89	(0.28-2.82)	0.8
Umbilical artery catheter	1.2	(0.26-5.9)	0.8	0.34	(0.11-3.7)	0.6	0.60	(0.10-3.63)	0.6
Invasive mechanical ventilation	5.3	(1.6-17.7)	0.007	3.4	(1.2-9.9)	0.02	3.4	(1.1-10.4)	0.03
Blood tests									
Number, mean	1.02	(0.98-1.07)	0.3	0.99	(0.94-1.0)	0.8	0.99	(0.94-1.04)	0.7
Phototherapy									
None	1	(reference)		1	(reference)		1	(reference)	
1-2 days	0.48	(0.19-1.2)	0.1	0.50	(0.19-1.3)	0.1	0.52	(0.19-1.4)	0.2
3-6 days	3.3	(1.1-9.6)	0.03	2.2	(0.73-6.6)	0.2	2.5	(0.71-8.4)	0.2
Apnoeas requiring stimulation									
Number, mean	1.0	(0.90-1.1)	1.0	0.97	(0.87-1.1)	0.6	0.97	(0.87-1.1)	0.6
Type of stimulation									
Gentle stimulation	1.47	(0.63-3.43)	0.4	0.82	(0.31-2.2)	0.7	0.81	(0.32-2.1)	0.7
Moderate stimulation	0.54	(0.14-2.02)	0.4	0.24	(0.05-1.1)	0.07	0.23	(0.05-1.1)	0.07
Funnel/facial O_2	0.92	(0.42-2.02)	0.8	0.43	(0.16-1.2)	0.1	0.44	(0.17-1.1)	0.09
Radiography	0.86	(0.32-2.3)	0.8	0.53	(0.20-1.4)	0.2	0.41	(0.12-1.40)	0.2
Head ultrasound scan	2.5	(1.1-5.8)	0.03	1.4	(0.38-5.0)	0.6	1.33	(0.33-5.26)	0.7

	Unadjusted		p-value	Adjusted for gestational age		p-value	Adjusted for gestational age and length of stay		p-value
Other procedures	OR	95% CI		OR	95% CI		OR	95% CI	
Abdominal ultrasound	0.14	(0.01-1.7)	0.1	0.20	(0.02-2.2)	0.2	0.20	(0.02-2.08)	0.2
Enteral feedings									
Total number of feeds	1.0	(0.99-1.1)	0.2	1.0	(0.98-1.1)	0.3	1.02	(0.98-1.06)	0.3
Type of food									
Expressed breast milk	1.7	(0.50-5.6)	0.4	2.3	(0.56-9.4)	0.2	3.27	(0.68-15.82)	0.1
Breast milk	1.1	(0.37-3.2)	0.9	2.1	(0.67-6.4)	0.2	2.64	(0.70-9.92)	0.2
Human milk fortifier	1.6	(0.50-5.31)	0.4	1.4	(0.36-5.3)	0.6	1.44	(0.36-5.83)	0.6
Formula	0.34	(0.13-0.88)	0.03	0.50	(0.19-1.3)	0.2	0.29	(0.08-0.99)	0.05
Mode of feeding									
Nasogastric tube	0.61	(0.22-1.70)	0.3	2.5	(0.32-19.7)	0.4	2.44	(0.34-17.80)	0.4
Orogastric tube	1.48	(0.27-7.98)	0.7	0.29	(0.04-2.0)	0.2	0.29	(0.04-1.94)	0.2
Breast feeds	1.08	(0.37-3.12)	0.9	2.1	(0.67-6.35)	0.2	2.63	(0.70-10.0)	0.2
Bottle	0.43	(0.09-2.12)	0.3	0.64	(0.10-4.0)	0.6	0.60	(0.08-4.5)	0.6
Syringe	0.40	(0.11-1.38)	0.1	0.80	(0.21-3.1)	0.7	0.86	(0.17-4.4)	0.9
Cup	0.30	(0.03-3.25)	0.3	0.50	(0.05-4.6)	0.5	0.52	(0.05-5.1)	0.6
Weight									
Times weighed									
1	1	(reference)		1	(reference)		1	(reference)	
2	1.2	(0.21-7.0)	0.8	1.4	(0.41-4.8)	0.6	1.4	(0.39-4.9)	0.6
3	0.86	(0.17-4.3)	0.9	1.0	(0.29-1.7)	1.0	1.0	(0.29-3.6)	1.0
4	1.4	(0.17-11.1)	0.8	1.0	(1.13-7.8)	1.0	0.98	(0.12-8.1)	1.0
Average weight ◊									
g, mean	1.0	(1.0-1.0)	0.4	1.0	(1.0-1.0)	0.4	1.0	(1.0-1.0)	0.4
Type of bed									
Incubator	1.2	(0.34-4.4)	0.8	0.60	(0.13-2.8)	0.5	0.59	(0.12-2.9)	0.5
Cot	0.48	(0.14-1.6)	0.2	1.4	(0.40-5.1)	0.6	1.4	(0.39-5.0)	0.6
Changed from incubator to cot	0.30	(0.07-1.3)	0.1	0.54	(0.11-2.7)	0.5	0.55	(0.11-2.8)	0.5
Bed spaces									
5a	1.8	(0.28-10.9)	0.5	2.7	(0.29-24.2)	0.4	2.6	(0.25-27.5)	0.4

	Unadjusted		p-value	Adjusted for gestational age		p-value	Adjusted for gestational age and length of stay		p-value
Bed spaces	OR	95% CI		OR	95% CI		OR	95% CI	
6a	0.72	(0.20-2.5)	0.6	0.85	(0.21-3.5)	0.8	0.83	(0.19-3.7)	0.8
7b	1.0	(0.24-4.4)	1.0	4.1	(0.69-24.4)	0.1	4.3	(0.69-27.5)	0.1
7c	1.7	(0.56-5.2)	0.4	3.7	(0.99-13.5)	0.05	3.6	(0.95-13.6)	0.06
7d	0.76	(0.14-4.2)	0.8	0.90	(0.18-4.3)	0.9	0.87	(0.16-4.7)	0.9
9	0.79	(0.17-3.6)	0.8	0.43	(0.06-2.9)	0.4	0.35	(0.04-2.9)	0.3
10	0.51	(0.05-5.3)	0.6	0.31	(0.03-3.7)	0.4	0.28	(0.02-3.3)	0.3
11a	0.95	(0.31-2.9)	0.9	0.56	(0.19-1.7)	0.3	0.57	(0.19-1.7)	0.3
11b	1.4	(0.49-3.8)	0.5	0.88	(0.32-2.4)	0.8	0.92	(0.32-2.7)	0.9
11c	1.6	(0.34-7.6)	0.5	1.5	(0.43-5.2)	0.5	1.5	(0.43-5.1)	0.5
11d	1.7	(0.32-8.6)	0.5	4.0	(0.69-22.6)	0.1	4.2	(0.78-23.5)	0.1
Bed space changes									
0	1	(reference)		1	(reference)		1	(reference)	
1	0.41	(0.12-1.4)	0.2	0.49	(0.13-2.0)	0.3	0.49	(0.12-2.0)	0.3
2	0.68	(0.12-3.8)	0.7	1.2	(0.19-7.0)	0.9	1.2	(0.18-7.2)	0.3
Rooms									
5	0.45	(0.07-2.7)	0.4	0.75	(0.11-5.2)	0.8	0.75	(0.11-5.2)	0.8
6	1.2	(0.41-3.6)	0.7	1.8	(0.51-6.2)	0.4	1.8	(0.48-6.7)	0.4
7	0.58	(0.20-1.7)	0.3	1.2	(0.30-5.1)	0.8	1.2	(0.27-5.2)	0.8
11	1.6	(0.74-3.7)	0.2	1.2	(0.49-2.8)	0.7	1.4	(0.45-4.5)	0.6

Unadjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Adjusted analysis was not performed for variables that had no cases, or cell number <10. CPAP – continuous positive airway pressure; PICC – peripherally inserted central catheter.

* Composite includes antibacterials against which *S. capitis* has resistance: amoxicillin, gentamicin, fusidic acid, metronidazole, and cefotaxime.

† Infloran probiotics contains *Bifidobacterium bifidum* and *Lactobacillus acidophilus*.

‡ Micelle E is a vitamin E formulation given for prevention of retinopathy of prematurity.

§ Vitadol C contains vitamin A, vitamin D and vitamin C.

‖ Oral sucrose is given as a pain relief for minor procedures.

¶ Temperature instability was defined by a neonate's requirement of clothing changes or incubator temperature changes to regulate their temperature.

◊ Average weight was per 100g for the multivariate analysis.

5.6 Prospective study population

The prospective study progress report period includes 1 July 2017 through 28 February 2018. The admissions and total number of *S. capitis* colonised neonates for the prospective study report period is shown in Figure 5. The flow diagram of admissions and their eligibility for inclusion in the prospective study is shown in Figure 10. Of the 222 neonates admitted to the NICU during the report period, 29 (13.1%) were swabbed at least once. Of the 193 (85.8%) not swabbed, 164 (85.0%) did not meet the gestational age criterion for receiving a swab test, 13 (6.7%) did not receive a swab test for unknown reasons, 9 (4.7%) were discharged before receiving their scheduled swab test, and 7 (3.6%) were transferred to another hospital before receiving their scheduled swab test. Among the 29 neonates swabbed at least once, 20 (69.0%) were eligible for participation. Of the 9 (31.0%) neonates ineligible for participation, 5 (55.6%) were excluded on the basis of not being admitted for long enough to receive ≥2 swabs, and 4 (44.4%) because of a positive initial swab.

Of the 20 eligible neonates, informed consent was provided for 13 (65.0%). Among the 13 participating neonates, one (7.7%) was colonised with *S. capitis* and 12 (92.3%) were not colonised. Of the 7 (35.0%) neonates not included because of lack of consent, 6 (85.7%) were on the basis of the parents declining consent for the neonate's participation, and one (14.3%) because the neonate was transferred before the informed consent process could be completed. For all of the neonate participants their birth mother provided consent for their own participation.

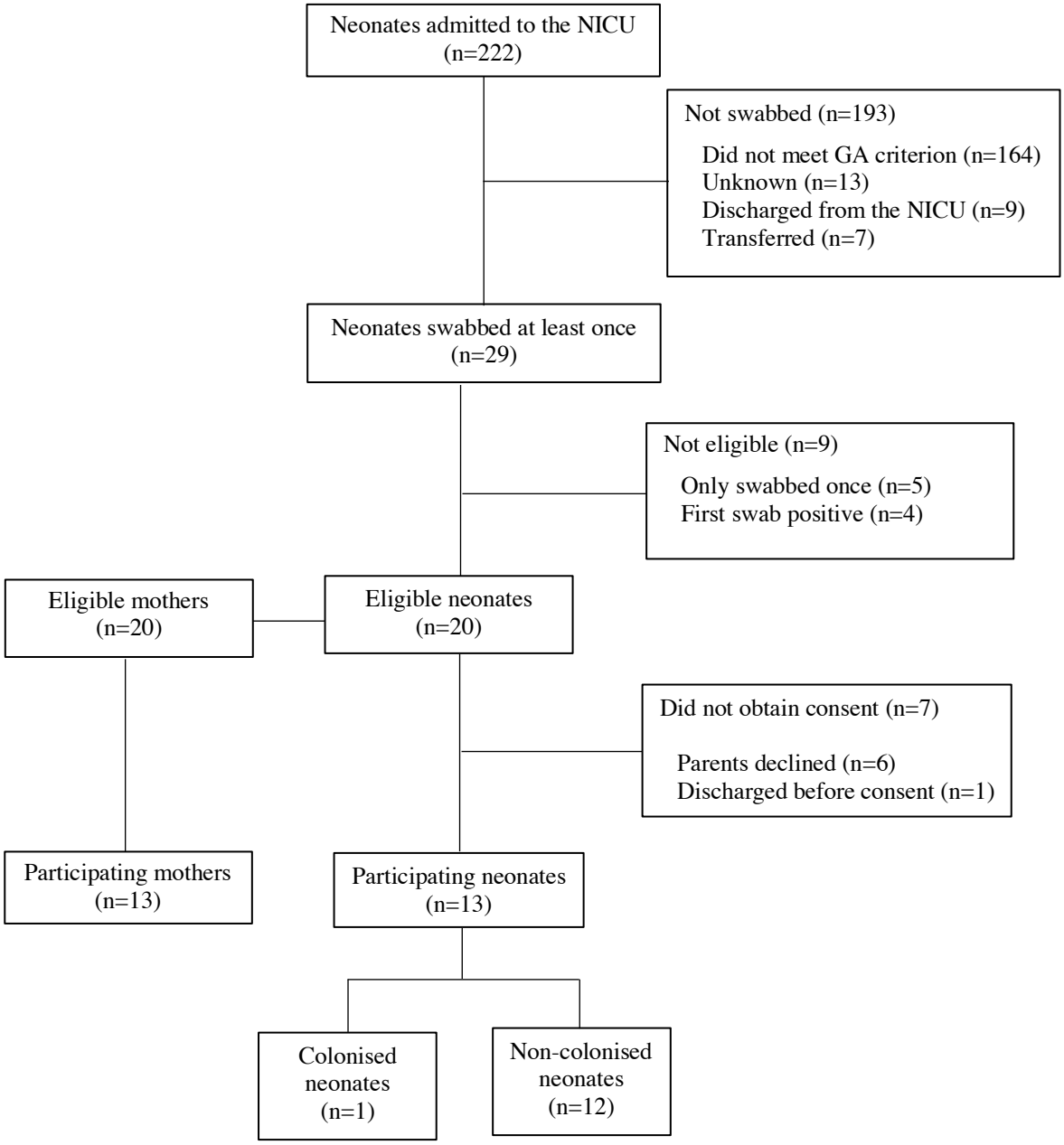

Figure 10: Flow diagram of the neonates and their eligibility of participation in the prospective study, Dunedin Hospital NICU, July 2017 through February 2018

NICU – neonatal intensive care unit; GA – gestational age.

5.6.1 Baseline characteristics of the study cohort

Table 18 shows the baseline characteristics for all participants, colonised, and non-colonised neonates during the prospective study report period. Among the 13 participants, 11 (84.6%) were New Zealand European, one (7.7%) was an 'other' ethnicity and one (7.7%) was supressed to protect confidentiality. The colonised neonate's gestational age was 27.9 weeks, while the mean (standard deviation) gestational age for non-colonised neonates was 31.0 (±0.8) weeks. The colonised neonate's birthweight was 1,075g and the mean (standard deviation) birthweight for non-colonised neonates was 1,427 (±122.8) g.

Table 19 shows the baseline characteristics of the mothers of all participants, colonised, and non-colonised neonates. The mother of the colonised neonates did not use antimicrobials during pregnancy. Among the mothers of the non-colonised neonates, 4 (33.3%) used antimicrobials during pregnancy. The mother of the colonised neonate had group B *Streptococcus* colonisation confirmed by positive combined vaginal and rectal swab. Among the mothers of the non-colonised neonates, none were positive for group B *Streptococcus*, 1 (8.3%) had chorioamnionitis and 1 (8.3%) had an infection during pregnancy. Among participants, 10 (76.9%) were the first live births for the mother participants.

Table 18: Baseline characteristics of all neonatal participants, colonised, and non-colonised neonates, Dunedin Hospital NICU, July 2017 through March 2018

	All participants n = 13		Colonised neonates n = 1		Non-colonised neonates n = 12	
Demographics	n	(%)	n	(%)	n	(%)
Sex						
Female	9	(69.2)	1	(100.0)	8	(66.7)
Ethnicity *						
New Zealand European	12	(92.3)			11	(91.7)
Māori	0	(0.0)			0	(0.0)
Samoan	0	(0.0)			0	(0.0)
Cook Island Māori	0	(0.0)			0	(0.0)
Tongan	0	(0.0)			0	(0.0)
Niuean	0	(0.0)			0	(0.0)
Chinese	0	(0.0)			0	(0.0)
Indian	0	(0.0)			0	(0.0)
Other: Kiribae/Tuvalu	1	(7.7)			1	(8.3)
Birth						
GA, weeks, mean (sd)	30.8	(±0.78)	27.9	(±0.0)	31.0	(±0.81)
Birthweight, g, mean (sd)	1,400	(±116.2)	1,075	(±0.0)	1,427	(±122.8)
Delivery type						
Caesarian section	10	(76.9)	1	(100.0)	9	(75.0)
Vaginal delivery tools						
Forceps	1	(7.7)	0	(0.0)	1	(8.3)
Ventouse	0	(0.0)	0	(0.0)	0	(0.0)
Multiple births	4	(30.8)	0	(0.0)	4	(33.3)
Born in Dunedin Hospital	12	(92.3)	1	(100.0)	11	(91.7)
In the NICU						
Length of stay, days, mean (sd)	NA		NA		NA	
Vital status at discharge						
Deceased, n (%)	0	(0.0)	0	(0.0)	0	(0.0)

NICU- neonatal intensive care unit; GA – gestational age; sd – standard deviation.
NA – did not collect date of discharge.
* Ethnicity was supressed for the case to protect confidentiality.

Table 19: Baseline characteristics of the mothers of all participants, colonised and non-colonised, Dunedin Hospital NICU, July 2017 through March 2018

	All participants n = 13	Colonised neonates n = 1	Non-colonised neonates n = 12
Current pregnancy			
Used any antimicrobials	4 (30.8)	0 (0.0)	4 (33.3)
Types of antimicrobials			
Metronidazole	2 (15.4)	0 (0.0)	2 (16.7)
Amoxicillin	1 (7.7)	0 (0.0)	1 (8.3)
Cephazolin	1 (7.7)	0 (0.0)	1 (8.3)
Erythromycin	1 (7.7)	0 (0.0)	1 (8.3)
Gentamicin	1 (7.7)	0 (0.0)	1 (8.3)
Medical history			
Cerclage	0 (0.0)	0 (0.0)	0 (0.0)
Chorioamnionitis	1 (7.7)	0 (0.0)	1 (8.3)
Gestational diabetes	0 (0.0)	0 (0.0)	0 (0.0)
IUGR	4 (30.8)	0 (0.0)	4 (33.3)
PROM	6 (46.2)	0 (0.0)	6 (50.0)
Group B *Streptococcus*	1 (7.7)	1 (100.0)	0 (0.0)
Premature delivery	13 (100.0)	1 (100.0)	12 (100.0)
Maternal infection	1 (7.7)	0 (0.0)	1 (8.3)
Family			
Number of other live births			
0	10 (76.9)	1 (100.0)	9 (75.0)
1	2 (15.4)	0 (0.0)	2 (16.7)
2	1 (7.7)	0 (0.0)	1 (8.3)

NICU- neonatal intensive care unit; IUGR – intrauterine growth restriction; PROM – premature rupture of membranes.

6 Discussion

Following adjustment for GA and length of stay, the retrospective study showed that premature neonates requiring oral NaCl supplementation, with a PDA, having CLD, or requiring invasive mechanical ventilation had greater odds for *S. capitis* colonisation. By contrast those who were members of a multiple birth, had inflamed skin, and who were formula fed had lower odds of *S. capitis* colonisation. Direct comparison of proportions of colonised with uncolonised neonates showed greater use of antimicrobials, including those to which the *S. capitis* strain was resistant, among colonised neonates. In addition, although individual bed spaces and rooms were not associated with increased risk for infection, examination of colonisation status of neonates over time within the Dunedin Hospital NICU demonstrates frequent new colonisation in the same room in consecutive weeks. This suggests that transmission may occur between neonates sharing the same room. Furthermore, *S. capitis*-colonised neonates are often moved rooms during the week that they become colonised. While the retrospective analysis had a number of limitations that will be addressed by the ongoing prospective study, on balance our results suggest that neonates requiring frequent contact due to more intensive medical management are at greater risk for colonisation.

Oral NaCl supplementation is used among neonates born at low GA to offset the leakage of sodium from the kidneys (141). The nested case-control study adjusted analysis showed that oral NaCl supplementation was associated with increased odds of *S. capitis* colonisation. A study by Al-Dahhan et al found that NaCl supplements in premature neonates improved their postnatal weight gain, with no undesirable side effects (142). However, they did not look at bacterial colonisation as an outcome of using NaCl. Oral NaCl use as a risk factor for CONS colonisation of neonates has not yet been investigated in any known published literature. Oral NaCl is likely a marker of the most premature, medically dependent, and frequently handled neonates. It is also plausible that high doses of oral NaCl could have an effect on the neonatal gut osmolality. Studies have shown that chronic high salt intake changed the bacterial count and the composition of the intestinal microflora in mice and increased the gut permeability (143, 144). Kloos et al found that 90% of *S. capitis* isolates were capable

of growth at NaCl concentrations of up to 10%. Therefore, it is possible that the change in the intestinal microflora induced by oral NaCl supplementation favours *S. capitis* colonisation (55).

The ductus arteriosus connects the pulmonary artery to the aorta and closes naturally within 4 days of birth for more than 95% of neonates with a birthweight >1,500g, but among neonates born ≤1,500g, only 34% of ductus arteriosus close within 4.3 days (145). Neonates with PDA represent members of a group of more medically dependent and frequently handled neonates for which *S. capitis* colonisation risk may be mediated by more frequent and prolonged contact than by the PDA pathophysiology itself. However, PDA has been associated with decreased blood flow velocity in the gut, which is associated with feed intolerance (146, 147). Neonates who have feed intolerance take longer to achieve full enteral feeds, which may affect the neonate's intestinal microflora (146). However, as neither the number of feeds per week or feed intolerance were significantly associated with colonisation the theory needs further investigation.

Both CLD and the requirement for invasive mechanical ventilation were associated with increased odds of *S. capitis* colonisation in the nested case-control adjusted analysis. Invasive mechanical ventilation is used to manage the breathing of neonates with respiratory distress syndrome (RDS) and CLD commonly resulting from bronchopulmonary dysplasia (BPD) (148), defined on the basis of the requirement for ventilation ≥28 days after birth (148). Furthermore, invasive mechanical ventilation is also a risk factor for lung injury and inflammation (149). Requirement of invasive mechanical ventilation is common among neonates born at <28 weeks' GA (149). Studies have shown that the use of non-invasive mechanical ventilation, such as nasal continuous positive airway pressure (CPAP), compared to invasive mechanical ventilation, is associated with a lower risk of nosocomial infections (150). In our adjusted analysis, we found that nasal CPAP was <1, although the association was not statistically significant, while invasive mechanical ventilation was associated with a statistically significant increased risk for *S. capitis* colonisation. It is possible that colonisation could occur from contaminated equipment. WGS of the Dunedin Hospital *S. capitis* NRCS-A strain showed that the *S. capitis* strain encodes the *ica*ADBC operon

for biofilm formation (section 2.4), therefore it is possible that the strain is able to persist on equipment and indirectly transmit to neonates (personal communication, James Ussher). If the ventilation equipment was contaminated it would colonise the skin or the gastrointestinal tract (GIT).

It is likely that oral NaCl supplementation, PDA, CLD, and invasive mechanical ventilation are markers of a subgroup of neonates with greater medical dependency whose risk factors for *S. capitis* colonisation are mediated by increased contact from staff and medical devices that are required for management of comorbidities. Premature neonates with complications require monitoring equipment and more regular checks by staff (personal communication, Roland Broadbent), with equipment such as stethoscopes that may be a source of *S. capitis* transmission in NICUs. *S. capitis* was isolated from stethoscopes and incubators in the Dunedin Hospital NICU between January 2014 through June 2014 (personal communication, James Ussher). Neonates with PDA, and CLD or invasive mechanical ventilation are also more likely to require acute care. In emergency situations, medical staff may need to attend neonates without standard precautions such as hand washing being possible, further increasing risk for *S. capitis* colonisation.

The nested case-control analysis showed that being born as a part of a multiple birth was associated with reduced odds of *S. capitis* colonisation. Studies have shown that neonates born as a part of a multiple birth are more likely to have similar microflora than non-multiple birth neonates (151, 152). One study found no difference between multiple birth neonates and singletons for episodes of LONS (151). Similar microflora between multiple birth neonates could be due to shared maternal contact, or identical maternal expressed breast milk (152). Additionally, neonates born as a part of a multiple birth may have contact with each other in the NICU, as shown in Figure 9 when one set of twins occupied the same cot, which does not occur between singletons. Members of the same twin pair regularly occupy the same room in the NICU, have shared equipment such as thermometers, and are cared for by the same staff members (personal communication, Roland Broadbent), which may facilitate transmission of *S. capitis*. One study found that a set of triplets had similar microflora regardless of two of the three being genetically identical (monochorionic monoamniotic) (152). Suggesting

that the retrospective study findings are more likely to be explained by common transmission, or lack thereof, than rather than protective genetic factors.

All multiple births in our retrospective study were twins, and all twin pairs were either both colonised or both non-colonised. The nested case-control analysis had eight cases that were part of a multiple birth. Of the eight cases, each twin became colonised with *S. capitis* within one week of the other (Figure 9). This observation suggests that transmission of *S. capitis* between twins is common. That being part of a multiple birth was protective against colonisation is likely an artefact of study design related to repeated baseline data among the controls due to risk set matching. At least one of the twins was in the NICU for one or more weeks as a control, and provided control data for themselves and their twin. This creates a bias towards multiple birth data being repeated among control data.

Adjusted analysis of the retrospective case-control study found that having an area of inflamed skin was associated with lower risk of *S. capitis* colonisation. In particular, inflamed axillary skin was protective against *S. capitis* colonisation after adjusting for GA and length of stay. To our knowledge, no epidemiologic studies have investigated skin inflammation as a risk factor for *S. capitis* or CONS colonisation of neonates. It is possible that skin inflammation, whether due to an infectious or non-infectious process, is hostile to *S. capitis*. However, there is presently little evidence to support this hypothesis. In the Dunedin Hospital NICU during the period of the retrospective study, persistently inflamed skin was often treated with topical fusidic acid, an antimicrobial against which the endemic *S. capitis* strain is resistant (personal communication, Roland Broadbent). While it is plausible that topical fusidic acid may simultaneously disrupt the protective effect of the normal skin and select for the endemic *S. capitis* strain, we were unable to perform an unadjusted or adjusted analysis as no cases used fusidic acid during the exposure week. Therefore, estimates were too small to interpret or the model did not converge so no estimates were available. The role of topical fusidic acid and other antimicrobial use is being examined in the ongoing prospective study.

Neonates enrolled in the retrospective study were fed with breast milk, expressed breast milk, or formula. Human milk fortifier was added to the breast milk or expressed breast milk for 6 (23.1%) cases and 55 (27.1%) controls. We found that enteral feeds with formula were associated with a reduced risk of *S. capitis* colonisation. Other studies have shown that it is possible for staphylococci to pass from the mother's expressed breast milk to the nose, throat, and bloodstream of neonates, with no evidence of mastitis in the mother (153), and that *S. capitis* has been isolated from human breast milk samples (85). The staphylococci could have transferred to the neonates through contaminated equipment associated with the storage of the expressed breast milk, such as pumps and bottles, or from the mother milk itself (153). Alternatively, breast feeding represents a period of extended direct skin contact with the mother that does not occur during formula or expressed breast milk feeding. Therefore, it is plausible that neonates fed with formula are protected against colonisation from bacteria in expressed breast milk, or from the maternal breast and skin. Against that, breast milk feeding was not associated with increased odds of colonisation in the adjusted analysis.

We had hypothesised that antimicrobial exposure might be associated with *S. capitis* colonisation due to its impact on the gut microflora (154, 155) and the evidence from other studies that altered microflora was a risk factor for LONS (155), including LONS due to vancomycin resistant *S. capitis* (16). In addition, WGS of the Dunedin Hospital *S. capitis* NRCS-A isolate showed that the strain was resistant to penicillin, tobramycin, fusidic acid, and had reduced susceptibility to chlorhexidine (section 2.4). Therefore, it is possible that exposure to these antimicrobials may select for *S. capitis* colonisation among neonates. However, while antimicrobial use, including use of antimicrobials to which the endemic *S. capitis* strain was resistant, was more common among colonised than non-colonised neonates, no significant difference in antimicrobial use was seen in either the unadjusted nested case-control analysis or after adjusting for GA and length of stay. This finding is consistent with antimicrobial requirements being greater among the most premature neonates than those born at later GAs, and suggests that antimicrobial exposure may not be the major the major driver of colonisation risk in the NICU setting. However, it is possible that antimicrobials were a risk factor for *S. capitis* colonisation but were not detected because the one week exposure period was

too short to detect an effect. An analysis with longer exposure periods may be possible in the future.

Location in a particular room or bed space during the exposure week was not associated with increased odds of *S. capitis* colonisation in the nested case-control adjusted analysis. However, the diagram of the rooms and bed spaces (Figure 8) showed that *S. capitis* colonisation regularly occur in consecutive weeks in the same room. For example, in room 11 a different neonate had a positive test result for *S. capitis* each week for four weeks. Certain rooms in the Dunedin Hospital NICU, such as room 11, are occupied by the most fragile neonates, and neonates in the same room oftern share equipment and nursing staff (personal communication, Roland Broadbent). There were fewer *S. capitis* positive swabs across consecutive weeks in other rooms, such as room 5, although the incidence rate of *S. capitis* positive neonates per study neonate-week per room was lower in room 5 compared to room 11. As neonates moved bed spaces frequently throughout their stay in the Dunedin Hospital NICU (personal communication, Roland Broadbent), and I did not collect data on bed spaces occupied by cases after they became positive so I was unable to investigate possible transmission to negative neonates in the same room after the case's exposure week.

6.1 Strengths and Limitations

A strength of our study design for both the retrospective and prospective studies was that we were able to establish temporality between exposure and outcome. We designed our eligibility criteria to establish exposure weeks during which colonisation status was known. Although having two axillary swab tests were necessary to investigate risk factors of colonisation, this eligibility criterion reduced our retrospective study sample size by half. Additionally, the medical records of almost half of our eligible neonates were stored in Dunedin Hospital storage rooms that were contaminated with asbestos. There was no way to access these records. The number of colonised neonates excluded from the retrospective study nested case-control analysis, included 16 neonates who were positive at their first swab and 16 whose weekly data were unavailable. Our nested case-control analysis had small numbers of neonates and therefore wide confidence intervals and concern for type II error, meaning the study may have failed to

detect true associations. Conversely, we examined many exposure variables, which would lead to inflation of the Type I error and a risk of false positive findings. We have not made formal adjustments for the multiple comparisons, but the results should be interpreted accordingly. The threshold for statistical significance was set at 0.05, so for one test there was a 5% chance the finding is a false positive association. With the large numbers of repeated statistical tests it is highly likely that there will be at least one false positive finding.

Our retrospective study showed differences between the eligible neonates and the ineligible neonates in terms of ethnicity, GA, birth weight, length of stay, and multiple births. Of the 119 ineligible neonates, 88 (73.3%) were not included because they were only swabbed once during their stay in the NICU, therefore were likely discharged or transferred within 14 days. This may have introduced selection bias as the relationship between the risk factors and the outcomes for the ineligible group are unknown, so may have been different than among the eligible neonates. Excluding neonates due to positive first swab is less likely to have caused selection bias, as they are more likely to be similar to the eligible neonates who were at risk of *S. capitis* colonisation. If there were bias, the estimated odds ratios in the nested case-control analysis would have been further from the null than the true value. Furthermore, as the ineligible neonate's relationship with exposures is unknown, and we did not investigate exposures before the first swab in our study, the results from the retrospective study may not be generalisable to any neonates that were only swabbed once.

Fortunately, we were able to collect baseline data for the neonates with unavailable weekly data using the electronic records on the Health Connect South online database. There were few differences between the neonates with available and unavailable weekly data in terms of ethnicity, multiple birth, and length of stay. It is unlikely that the relationship between treatment, co-morbidities, or staff contact and *S. capitis* colonisation for neonates with available weekly data was different from those with unavailable weekly data as their GA and birthweight were similar.

Information bias in a retrospective study can occur from how the data were recorded and how the data were collected. Different professionals including nurses, consultants,

registrars, and allied health professionals recorded data in the neonate's medical records. Within in each profession there were numerous individuals, which resulted in variation between what was recorded in the records. Data were only available from the medical records if they had been recorded. It was not always possible to determine whether the absence of information reflected unknown exposure or no exposure. Data such as antimicrobial usage and procedures were reliably recorded in the medical records, while data such as parental contact and other transmission data were recorded in a more haphazard manner. It is possible that there was some misclassification of exposures in our study due the data available in the medical records. Healthy neonates could have less recorded on their medical records, as the details of their condition may not be as important as it is for the fragile neonates. If this occurred, the misclassification of exposures would have been differential and caused the estimated values to be further from the null than the true values. Alternatively, bias could have been non-differential as it could have been the type of data collected that was unreliable, rather than a difference between the cases and controls. This would have caused the estimated values to be closer to the null than the true values.

To reduce the potential effect of information bias from how data were collected, I created the CRF to have as few free-form questions as possible so the measures of exposure were standard. The majority of our weekly CRF questions required 'yes' or 'no' responses, and the definitions for the medical history, procedures, and devices were not flexible. Additionally, I was the coordinating investigator of the retrospective study, and performed all the data collection and management, so there was minimal variation of responses between neonates.

Blinding the person performing data collection to the objectives of the study and the outcomes of each neonate can reduce information bias. There was no practical way that I could have been blinded to the neonate's outcome at the time of retrospective data collection. I stopped collecting data for each neonate when they either were discharged from the NICU, did not receive their scheduled swab test, or became positive for *S. capitis* colonisation. As almost half of the cases became positive of *S. capitis* in their first full week in the NICU, and none became positive in their last week in the NICU, being blinded to the outcome would have meant unnecessary data collection for weeks

after data was no longer required. Recruiting another person to perform data collection would have required substantially more resources. Blinding of the outcome is addressed in the prospective study, as the weekly *S. capitis* test result becomes available after the weekly CRF is completed.

As the *S. capitis* swab test was performed every Monday morning, and the exposure week was Monday 5.59am to Monday 6am, the collection of exposures differed depending on the day of the week the neonate was born. For example, almost all low GA neonates who are admitted to the Dunedin Hospital NICU are treated with amoxicillin and gentamicin for 2-3 days after birth (personal communication, Roland Broadbent). However, this is not apparent in our results (Table 8) because if a neonate were born from Tuesday through Thursday for example, their antimicrobial treatment would not have been recorded in their first CRF. Additionally, neonates are most dependent during their first week in the NICU so other treatments such as ventilation may also have been missed from the first CRF. This could have caused non-differential misclassification of exposures. Additionally, the exposure week was an arbitrarily selected duration. As approximately half of the cases in the retrospective study became positive in their first full week in the Dunedin Hospital NICU, we know that colonisation can occur within a short period. However, some exposures may take longer for skin colonisation to be detected. For example if colonisation begins in the GIT, it may take longer for *S. capitis* to colonise the skin. We are planning to explore the nested case-control analysis with longer exposure periods in the future.

The diagnostic test used to confirm *S. capitis* NRCS-A identification (section 4.3) has high specificity because it will only give a positive result if *S. capitis* is present in the sample. However, the sensitivity of the *S. capitis* axilla swab test is unknown. The NICU staff do not have a protocol for size of axilla surface area swabbed, swab pattern, or if one or both axillae are swabbed. Furthermore, as discussed in the literature review (section 2.1.1), the most populated sites of neonates, and the preferred niches of CONS species, are unknown. We cannot be certain that the axilla was the most appropriate site to identify colonisation, stool samples may be more appropriate than skin samples. If the test had low sensitivity, or the site of the test did not support the highest populations of *S. capitis* growth, there may have been misclassification of the outcome.

The controls were matched to the cases as described in the methods (section 4.17.3). We decided to match controls to cases to reduce the confounding, based on a hypothesis that the risk of *S. capitis* transmission between neonates that could fluctuate over time depending on the number of *S. capitis* positive neonates in the NICU. We planned to match the controls to cases using the exposure week only; however, there were not enough controls to match to each case, which would have reduced the number of cases in our study. We then matched the controls to cases using the exposure week, and three weeks previous, which gave us controls for every case. However, this method meant that an individual neonate could have multiple weeks of their control data matched to an individual case. Additionally, an individual neonate could have their own control data matched to their case data. This meant that in 14 (53.8%) of 26 risk sets, there were correlations between weeks which standard conditional logistic regression does not allow for, and from the resulting analysis, the p-values would likely be too small. I used robust standard errors within the conditional logistic regression, which will have accounted for this correlation to some extent.

Matching reduced the number of non-colonised neonates who contributed control data. The small numbers and nested nature of the study design resulted in repeated baseline and weekly data for individual neonates, some contributing as many as 21 weeks of control data. Weekly data varied between study weeks more frequently than baseline data. If a neonate contributed twice as a control, their baseline data would be the same each time. The effect of this repetition in the baseline data is evident when the numbers and proportions between the cases and controls that provided weekly data (Table 5) are compared with the cases weeks and control weeks (Table 6). The neonates who contributed the most control data were those of low GA and low birthweight who were in the NICU for longer periods and therefore had more opportunity to match with cases on time, although we adjusted for this in the nested case-control adjusted analysis. Furthermore, colonised neonates contributed approximately 60% of the control data compared to non-colonised neonates who contributed approximately 40% (results section 5.3).

We controlled for confounding of GA and length of stay in our adjusted analysis. Therefore, there may have been confounding from factors that were unmeasured or for

which our sample size was too small to include. We were unable to perform a multivariate analysis as our sample size was too small and would have produced unreliable results.

We used categorical variables for the adjusted analysis as it was not likely that GA and length of stay fit a linear relationship with *S. capitis* colonisation. If GA fit a linear relationship, every week increase in GA would reduce the log odds of *S. capitis* colonisation. This may be applicable at low GA, but may not be applicable closer to term. Therefore, there may have been residual confounding from the use of categorical variables rather than continuous variables in the adjusted analysis.

6.2 Recommendations

The increased risk of *S. capitis* colonisation associated with comorbidities and devices that may require more staff contact suggests that staff play a role in transmission. If this is the case, the standard precautions for infection prevention and control used by the Dunedin Hospital NICU may not be adequate for preventing *S. capitis* colonisation. The Dunedin Hospital NICU could strengthen their precautions using an education programme to improve hand hygiene practices, which has shown to work in another hospital ICU (91). Alternatively, the Dunedin Hospital NICU may want to consider transmission-based contact precautions (section 2.1.4). Cohorting neonates would require one or more dedicated rooms in the NICU for all colonised neonates, with non-colonised neonates in separate rooms with standard and transmission-based precautions between these separate spaces. Ideally, staff who care for colonised neonates would not have contact with non-colonised neonates. Table 16 and Figure 8 show that 6 (23.1%) of 26 cases moved bedspaces during the exposure week. As neonates become colonised during the exposure week, unknown to staff until the next swab result, reducing the movement of neonates around the NICU would reduce the number of other neonates at risk.

We identified the neonates most at risk of *S. capitis* colonisation as those born at low GA, low birthweight, and those with comorbidities. The NICU should continue to focus surveillance and interventions on preventing transmission of *S. capitis* to this group of

neonates. However, our retrospective study also showed that two neonates born at >36 weeks' GA became colonised, and therefore colonised high GA neonates may be missed by prospective study surveillance. As two neonates is a small number increasing the surveillance to all neonates may not be a reasonable use of resources, but the NICU should be aware of the possible role of the higher gestational age and birthweight neonates in transmission.

I recommend that the prospective study continues until the sample size is large enough for adequate power in the study analysis (section 4.9). The Dunedin Hospital *S. capitis* NRCS-A strain encodes genes for biofilm formation (section 2.4), and has been isolated from incubators and stethoscopes in the NICU (section 2.4). It is possible that transmission to neonates occurs indirectly through fomites, however, we were unable to trace the use of incubators, cots, or stethoscopes in the retrospective study. As discussed in section 2.1.3, the clonality of *S. capitis* NRCS-A suggests global dissemination through either movement of healthcare workers or through a point source outbreak of contaminated NICU equipment, although none has been identified. The prospective study incorporates improvements from the retrospective study including available weekly data for all participants, tracing Dunedin Hospital NICU staff members, and tracing equipment such as incubators and stethoscopes. We hope to repeat analyses for the associations we found for oral NaCl use, PDA, CLD, invasive mechanical ventilation, multiple birth, skin inflammation, and enteral feeds with formula. Additionally, we hope to explore the role of antimicrobial use on risk of *S. capitis* colonisation. The prospective study has potential for transcription bias as the investigator of the study entering the data from the CRFs into the REDCap online database. It was not possible to perform double entry of the data due to our resources, however if the study continues as recommended, double entry of the data should be considered.

Figure 8 shows that there were consecutive weeks of cases in room 11, for four weeks in a row. This suggests that being in the same room as a case during their exposure week may be risk factor for colonisation. To explore this concept further we would need to identify for each week of stay if a neonate was in the same room as a case or a control. However, as our study was not designed to investigate infant-infant

transmission, we do not have sufficient data for this investigation. We do not have the weekly data for half of the participants due to asbestos storage, the neonates who had a positive first swab, or the colonised neonates after they become positive, all of which are necessary for an analysis on transmission. For the prospective study, we collect the bed space number and colonisation status of neonates cared for by the same nurse as participants. Due to findings in our retrospective study, the NICU now records the incubator identification number on the NICU category 4 or 5 chart in the neonate's medical records. During analysis of the prospective study, the incubators will be able to be traced. For the prospective study the incubator and bed space numbers should be recorded for the neonate's entire stay, regardless of outcome, so that they can be traced to determine transmission among neonates and among rooms.

Studies need to be done to confirm the sensitivity of the swab test. This is important to understand the misclassification of the outcome, if any, in the studies. Additionally, a longitudinal study with a large sample size looking at the site of neonates most populated with *S. capitis* is important. Six studies investigated which body sites of premature neonates had high CFU/area of CONS but they had small sample sizes and there was discordance of the sites swabbed (Table 1). Additionally, these studies were conducted from 1989-1996, and the predominance of *S. capitis* on neonates has changed in recent years (8). Such a study would be helpful in understanding if *S. capitis* has a niche environment or if it is spread over the whole body. The prospective study trialled testing neonates stool samples for colonisation of *S. capitis*. It is important to continue stool collection, especially with increasing evidence that CONS heavily colonise the GIT and could enter the bloodstream through bacterial translocation.

6.3 Conclusions

We conducted a retrospective study to investigate the risk factors for *S. capitis* NRCS-A colonisation among premature neonates in the Dunedin Hospital NICU. The retrospective study showed that oral NaCl use, having a comorbidity such as PDA or CLD, and requiring invasive mechanical ventilation were associated with increased risk of *S. capitis* NRCS-A colonisation. Although we had insufficient data for a full multivariate analysis, and the results were solely based on the retrospective study which is more likely than a prospective study to include bias, we hypothesised that neonates with comorbidities are likely the most premature, medically dependent neonates who require increased contact from Dunedin Hospital NICU staff or equipment. Our results suggest that the Dunedin Hospital NICU may need to strengthen their standard precautions for infection prevention and control or consider transmission-based contact precautions, such as cohorting neonates, to prevent transmission of *S. capitis* NRCS-A. In order to further understand the risk factors of *S. capitis* NRCS-A colonisation among neonates in the Dunedin Hospital NICU, we recommend that the findings from the retrospective study, along with the use of antimicrobials, and tracing contact from healthcare workers and the NICU environment be explored with an ongoing prospective study.

7 References

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8 Appendices

Appendix 1: Prospective study *S. capitis* test result CRF

Case Report Form Participant ID: _ _ _

Case Report Form – Test results

Today's date (dd/mm/yyyy): ___ /___ /_____

Person completing form (*first name and surname*): _____

Section 1: Axilla swab test results.

1. What is the date of the axilla swab collection? (*dd/mm/yyyy*) __ / __ / _____
2. What is the *S. capitis* test result from the axilla swab?
   ☐ Positive ☐ Negative

Appendix 2: Prospective study baseline CRF

Case Report Form Participant ID: _ _ _

Case Report Form – Baseline

Today's date (*dd/mm/yyyy*): ___ /___ /_____

Person completing form (*first name and surname*): _____

General guidelines for filling out the Baseline case report forms.

- If there is a yes/no option, make sure you tick one, do not leave unanswered.
- If 'yes' is ticked for 'other' you must specify the procedure, equipment or medication etc. that was not provided in the list.
- Pay attention to the time frames (either 24 hours or 7 days). Do not include anything that happened outside that time frame (e.g. nothing after 6am today).
- Records are 6am - 6am (follows the shifts).
- **'7 days'** refers to the previous Monday at 6.00am, to Monday (today) at 5.59am.
- **'24 hours'** refers to the final 24 hours of the 7 day period i.e. Sunday (yesterday) 6.00am to Monday 5.59am. When recording the date for the last '24 hours' use Sunday's date (yesterday's date).

Section 1: Information from Neonatal Records

A. Demographic information - Infant

1. Sex (*must tick one*): ☐ Male ☐ Female

2. Date of birth (*dd/mm/yyyy*): ___ /___ /_____

3. Date of admission (*dd/mm/yyyy*): ___ /___ /_____

4. Gestational age at birth (*fill in the number of weeks as well as the additional days*): ____ weeks ____ days

5. Birth weight: _____ grams

6. Infant ethnicity (*tick all that apply*):
☐ NZ European ☐ Māori ☐ Samoan ☐ Cook Island Māori ☐ Tongan
☐ Niuean ☐ Chinese ☐ Indian ☐ Other: _____

7. Does the neonate have siblings from other births? ☐ Yes ☐ No

If 'yes', number of living siblings from other births (*e.g. 01, 02, 03*): ____

B. Birth history

1. Delivery type (*must tick one*): ☐ Caesarean section ☐ Vaginal delivery

If the infant was born by vaginal delivery, were any of the following included?
(*tick all that apply*): ☐ Forceps ☐ Scalp electrodes ☐ Ventouse ☐ None apply

2. Did this pregnancy involve multiple births? ☐ Yes ☐ No

If 'yes', how many births? (*e.g. triplets = 3*): ☐ 2 ☐ 3 ☐ 4 ☐ 5

Participant ID for other infant/s (*IDs are printed on the case report forms for the other participants)*: ___ ___ ___ ___ , ___ ___ ___ ___ , ___ ___ ___ ___

3. Was the infant born in Dunedin Hospital: ☐ Yes ☐ No

If 'no', where was the infant born? (*the closest town or city*): _____

If 'no' (*select only one*): ☐ Health care facility ☐ Other (*e.g. at home*): _____

If 'yes' to health care facility, what facility? (*e.g. hospital, birthing facility*):
_____

Case Report Form – Baseline

Today's date (*dd/mm/yyyy*): ___ /___ /_____

Person completing form (*first name and surname*): _____

Section 2: Information from maternal records and maternal recall

We need to have consent for the mother's participation and to collect maternal records.

A. Demographic information – Mother

Information in section A can be collected by asking the mother.

1. Mother's date of birth (*dd/mm/yyyy*): ___ /___ /_____

2. Mother's ethnicity (*tick all that apply*):
☐ NZ European ☐ Māori ☐ Samoan ☐ Cook Island Māori ☐ Tongan
☐ Niuean ☐ Chinese ☐ Indian ☐ Other: _____

B. Maternal antimicrobial use

Information in Section B can be collected from the mother's medical records and/or by asking the mother.

1. Was the mother treated with antimicrobials during this pregnancy? ☐ Yes ☐ No
   If 'yes', fill out the following table.

IV – intravenous, PO – per oral, PR –per rectal

Antimicrobial (*generic name*)	Route (*IV, PO, PR, topical*)	Start date (*dd/mm/yyyy*)	Stop date (*dd/mm/yyyy*)

C. Maternal history of current pregnancy

Information in Section C can be found in the infant's notes, maternal medical records, and by asking the mother. More than one source may be needed to collect data for this question.

1. Complications during this pregnancy.

Tick either yes (Y) or no (N) for each of the following (do not leave blank).

Y N

☐☐ Cerclage
☐☐ Chorioamnionitis
☐☐ Gestational diabetes
☐☐ Intrauterine growth restriction (IUGR)
☐☐ Premature rupture of membranes (PROM)
☐☐ Other: _____
☐☐ Group B Strep colonisation
☐☐ Premature delivery
☐☐ Premature delivery

Appendix 3: Prospective study weekly CRF

Case Report Form Participant ID: _ _ _

Case Report Form – Weekly

Today's date (dd/mm/yyyy): ___ /___ /_____

Person completing form (*first name and surname*): _____

General guidelines for filling out the Weekly case report forms.

- If there is a yes/no option, make sure you tick one, do not leave unanswered.
- If 'yes' is ticked for 'other' you must specify the procedure, equipment or medication etc. that was not provided in the list.
- One day is recorded from 6am to 6am (follows the charts).
- Pay attention to the time frames (either 24 hours or 7 days). Do not include anything that happened outside that time frame (e.g. nothing after 5.59am today).
- **'7 days'** refers to the previous Monday at 6.00am, to Monday (today) at 5.59am.
- **'24 hours'** refers to the final 24 hours of the 7 day period i.e. Sunday (yesterday) 6.00am to Monday 5.59am. When recording the date for the last '24 hours' use Sunday's date (yesterday's date).
- For dates in most of the tables you can write 'ongoing' if the dates are outside the 7 day period. E.g. if the infant has been in the same incubator before and after the 7 day period, write 'ongoing' in both start and stop date columns.
- For parental contact: if there are multiple Mothers/Fathers use A for genetic parent and B for partner.

Section 1: Information from case notes

Information will be found in the infant's case notes. Ask the nurses assigned to the infant if you are unsure about any of the notes.

A. Infant medical history

1. Comorbidities in the last **7 days** (*last Monday at 6am to today (Monday) at 5.59am)*

Tick either yes (Y) or no (N) for each of the following if the onset is in the last 7 days (do not leave blank).

Y	N		Y	N	
☐	☐	Sepsis workup	☐	☐	Renal impairment
☐	☐	Apnoea of prematurity	☐	☐	Retinopathy of prematurity (ROP)
☐	☐	Feed intolerance (feeds workup)	☐	☐	Sepsis diagnosis
☐	☐	Gastric aspirate (bile present)	☐	☐	Skin injury
☐	☐	Hypoglycemia	☐	☐	Area of inflamed skin
☐	☐	Neonatal encephalopathy	☐	☐	Umbilical flare
☐	☐	Patent ductus arteriosis	☐	☐	Flaky skin
☐	☐	Temperature instability			
☐	☐	Intracran. hemorrhage. *If 'yes' grade*: ☐ 1 ☐ 2 ☐ 3 ☐ 4			
☐	☐	Cardiac abnormalities (*e.g. congenital heart disease*): _____			
☐	☐	Pulmonary disease (*e.g. CLD, HMD/RDS, meconium aspiration*): _____			
☐	☐	Gastrointestinal disease (*e.g. NEC, gastroschisis, omphalocele*): _____			

2. Did the infant have any of the following procedures or devices *in situ* in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)*

Tick either yes (Y) or no (N) for each of the following if the procedure was in the last 7 days (do not leave blank).

Y	N		Y	N	
☐	☐	Endotracheal (re)intubation, #: ____	☐	☐	Supplemental O_2
☐	☐	Invasive mechanical ventilation	☐	☐	Retinopathy of prematurity screen
☐	☐	Nasal CPAP	☐	☐	Umbilical Artery Catheter (UAC)
☐	☐	Nasal cannula (Optiflow)	☐	☐	Umbilical Vein Catheter (UVC)
☐	☐	Peripheral IV	☐	☐	Peripheral Inserted Central Catheter
☐	☐	Orogastric tube			
☐	☐	Nasogastric tube			
☐	☐	Blood tests. *If 'yes,'* total number: _____			
☐	☐	RBC transfusion. *If 'yes,'* total number: _____			
☐	☐	Phototherapy. *If 'yes,'* duration: _____ hours (*total*), and bili blanket: ☐ Yes ☐ No			
☐	☐	Syringe pump. *If 'yes,' tick all that apply*: ☐ inside incubator ☐ outside incubator			
☐	☐	Other devices (*must include if the device is inside or outside the incubator*): _____			

_____

If 'yes' to invasive mechanical ventilation, nasal CPAP or nasal cannula, record the ventilator asset number (*asset number only*): _____

3. Additional comments:_____
_____
_____
_____

B. Medication history

1. Was the infant treated with any antimicrobials in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No
   - *If 'yes', fill out the following table.*

IV – intravenous, PO – per oral, PR –per rectal
Start date/ongoing - If the infant started receiving the treatment before the 7 days period begun, write 'ongoing'
Stop date/ongoing - If the infant is still receiving the treatment write 'ongoing'

Antimicrobial	Route (*IV, PO, PR, topical*)	Start date/ongoing (*dd/mm/yyyy*)	Stop date/ongoing (*dd/mm/yyyy*)

2. Other medications, excluding injectables, received in the last **7 days**. (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No
   - *If 'yes', fill out the following table.*

IV – intravenous, PO – per oral, PR –per rectal
Start date/ongoing - If the infant started receiving the treatment before the 7 days period begun, write 'ongoing'
Stop date/ongoing - If the infant is still receiving the treatment write 'ongoing'

Medication	Route (*IV, PO, PR, topical*)	Start date/ongoing (*dd/mm/yyyy*)	Stop date/ongoing (*dd/mm/yyyy*)

3. Other injectables received in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No
   - *If 'yes', fill out the following table.*

Product example - TPN (Total Parental Nutrition), Lipids, 10% dextrose, insulin.
Start date/ongoing - If the infant started receiving the treatment before the 7 days period begun, write 'ongoing'
Stop date/ongoing - If the infant is still receiving the treatment write 'ongoing'

Product	Start date/ongoing (*dd/mm/yyyy*)	Stop date/ongoing (*dd/mm/yyyy*)

4. Additional comments:_____
_____
_____
_____

C. Parental/family contact

Contact refers to any physical contact with the infant, inside or outside the incubator.

1. Did the neonate have any contact with its parents or guardians in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No

If 'yes', fill out the following table.

For 'contacts' ONLY use: 'Mother', 'Father' or 'other family member'.
Duration: short – 0-14 minutes, medium – 15-29 minutes, long – 30+ minutes.
Do not include contact from cares – there is a section for this later in the form.

Contacts (Mother, Father or other family member)	Contact type (*e.g. skin to skin, hand touch, clothed cuddle*)	Duration (*short, medium, long*)	Dates (*dd/mm/yyyy*)

2. Comment on how much family contact (*mother, father or other family member*) there was in the last **24 hours** (*a little, medium, a lot*): _____

3. Did the infant have contact with its twin/multiple in the unit in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No

Participant IDs are printed on the case report forms for the other participants

Other infant's participant ID	Type of contact (*skin to skin*)	Duration (*short, medium, long*)	Dates (*dd/mm/yyyy*)

4. Additional comments:_____
_____
_____
_____

D. Enteral feedings

The last 7 days: (*last Monday at 6am to today (Monday) at 5.59am)*

Infants are usually fed at regular intervals throughout the day. If fed regularly multiply the number of feeds per day by 7. (*e.g. 2 hourly feds = 12 feeds per day, therefore they would be fed 84 times in the last 7 days*).

1. How many times was the infant fed in the last 7 days? _____

If the feeding routine changed in the last 7 days, was it a routine change (e.g. specific date the routine changed)? *Tick either*: ☐ Routine change ☐ Irregular change to routine

If 'routine change' was ticked, record the date that the feeding routine changed.
(*dd/mm/yyyy*) ___ /___ /_____

2. Was the infant fed donor human breast milk in the last 7 days? ☐ Yes ☐ No

What type of donor human breast milk was used in the last 7 days? (*select one or both*)
☐ Pasteurised, *total number of feeds*: _____
☐ Frozen screened, *total number of feeds*: _____

The last 24 hours: (*Sunday 6am – Monday 5.59am, use the date for Sunday*)

3. How many times was the infant fed breast milk in the last 24 hours?
(*e.g. 01, 02, 03*) ____

Fill in the following table if the infant was fed breast milk in the last 24 hours.

Date – there will only be one date (last 24 hours- yesterday's date). Please record it in the top row.
Type - Fortified Human Breast Milk (FHBM) or Mothers Breast Milk (MBM), do not include donor breast milk.
Number of feeds– Number of the same type and route of feed in the last 24 hours (if all feeds were the same, this will be the same as the total feeds for the last 24 hours).

Date (*dd/mm/yyyy*)	Type *(FHBM or MBM)*	Route (*breast, bottle*)	Duration (*minutes- per feed*)	Number of feeds (*per route and type*)

4. How many times was the infant fed formula in the last 24 hours? *(e.g. 01, 02, 03*) ____

If the infant was fed formula (*tick all that apply*):
☐ Ready To Feed (RTF) ☐ Mixed by staff (MBS)

Fill in the following table if the infant was fed formula in the last 24 hours.

Date – there will only be one date (last 24 hours- yesterday's date). Please record it in the top row
Type – ready to feed (RTF) or mixed by staff (MBS)
Number of feeds– Number of the same type of feed in the last 24 hours

Date (*dd/mm/yyyy*)	Type (RTF or MBS)	Duration (*minutes*)	Number of feeds (*per type*)

5. Additional comments:_____
_____
_____
_____

E. Environment

If the infant has changed bedspace/incubator/cot/stethoscope in the last 7 days please fill out both available rows on the table so we have a record of both bedspace numbers and when the change occurred. Make sure the dates are recorded.

The last 7 days: (*last Monday at 6am to today (Monday) at 5.59am)*

1. Bedspace number

Start date/ongoing - If the infant is in the same bedspace from before the 7 day period began, write 'ongoing'
Stop date/ongoing - If the infant is in the same bedspace (post 7 day period) write 'ongoing'

Bedspace number (*e.g. 01, 02, 03*)	Start date and time/ongoing (*dd/mm/yyyy, 24hr time*)	Stop date and time/ongoing (*dd/mm/yyyy, 24hr time*)

2. Incubator number (*only fill out the table if the infant is in an incubator*)

Start date/ongoing - If the infant is in the same incubator from before the 7 day period began, write 'ongoing'
Stop date/ongoing - If the infant is in the same incubator (post 7 day period) write 'ongoing'

Incubator number (*e.g. 01, 02, 03*)	Start date and time/ongoing (*dd/mm/yyyy, 24hr time*)	Stop date and time/ongoing (*dd/mm/yyyy, 24hr time*)

3. Cot number (*only fill out the table if the infant is in a cot*)

Start date/ongoing - If the infant is in the same cot from before the 7 day period began, write 'ongoing'
Stop date/ongoing - If the infant is in the same cot (post 7 day period) write 'ongoing'

Cot number (*e.g. 01, 02, 03*)	Start date and time/ongoing (*dd/mm/yyyy, 24hr time*)	Stop date and time/ongoing (*dd/mm/yyyy, 24hr time*)

4. What stethoscope number was used for the infant in the last **7 days**? _____

Were any other stethoscopes used for this infant in the last 7 days? ☐ Yes ☐ No

If 'yes', what is the number of the new stethoscope(s)? _____

Record the date(s) the stethoscope changed. *Record all dates, if the stethoscope has changed more than once* (*dd/mm/yyyy*): __ /__/ _____, __ /__/ _____, __ /__/ _____

5. Was the infant weighed in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No

Please fill out for every time the infant was weighed (usually three times a week)

Inbuilt or external scale	Weight (*grams*)	Date (*dd/mm/yyyy*)

6. Equipment used on the infant in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)*

Tick either yes (Y) or no (N) for each of the following (do not leave blank).

Y	**N**		**Y**	**N**	
☐	☐	Arterial line	☐	☐	Blood pressure (BP) cuff
☐	☐	Cardio/respiratory (CR) monitor	☐	☐	Electrocardiogram (ECG)
☐	☐	Leads	☐	☐	Neopuff (Ambubag)
☐	☐	Probes SaO_2	☐	☐	Pumps (any)
☐	☐	Suction	☐	☐	Tape measure
☐	☐	Thermometer			
☐	☐	Temperature probe			
☐	☐	Other: _____			

If 'yes' for thermometer, tick if it was the infants own thermometer or shared thermometer, and the asset number. *Tick one or both:* ☐ Own thermometer, asset number: _____
☐ Shared thermometer, asset number: _____

F. Infant cares

1. What is the number of normal cares in the last **24 hours**? (*e.g. 01, 02, 03*) _____
Cares over a 24 hour period is routine. If the infant needs cares every 3 hours, this will equal 08 cares

2. Used in cares in the last **24 hours**:

Tick either yes (Y) or no (N) for each of the following (do not leave blank).

☐	☐	CPAP change (prongs/mask)	☐	☐	Feeding tube change
☐	☐	Head-to-toe assessment	☐	☐	IV cannula change
☐	☐	Linen change (any)	☐	☐	Nappy change
☐	☐	Observations	☐	☐	Suction
☐	☐	Temperature assessment	☐	☐	Vent/CPAP circuit change
☐	☐	Washing/wipe			
☐	☐	Other: _____			

3. Record if there was any parental contact for cares in the last **24 hours**.
Contacts (*only use: 'Mother' or 'Father')*: _____
Type of cares (*e.g. for nappy change)*: _____

G. Apnoeas requiring stimulation

1. Has the infant had any apnoeas requiring stimulation in the last **24 hours**?
   ☐ Yes ☐ No

If 'yes,' number in incubator in the last **24 hours** (*e.g. 01, 02, 03*): _____

If 'yes,' which staff were involved (*tick all that apply*):
☐ Nurse ☐ Registrar ☐ Consultant

Intervention type (*tick all that apply*):
☐ Gentle ☐ Vigorous ☐ Bag and mask ☐ Neopuff (Ambubag)
☐ Other: _____

H. Other nursing procedures

1. Other nursing procedures in the last **24 hours** (*e.g. suppositories, urine collection*).
   ☐ Yes ☐ No

If 'yes', what procedure(s) (*list all*)? _____

If 'yes', total number of other nursing procedures in the last **24 hours** (*e.g. 01, 02, 03*): _____

I. Other procedures

1. Other procedures in the last **7 days** (*last Monday at 6am to today (Monday) at 5.59am)*.

Tick either yes (Y) or no (N) for each of the following (do not leave blank).

Y	**N**	
☐	☐	Abdominal ultrasound
☐	☐	Echo cardiogram
☐	☐	Eye check
☐	☐	Head Ultrasound Scan (HUS)
☐	☐	Scans
☐	☐	X-ray
☐	☐	Other: _____

J. Consultant and registrar contact

1. Fill out the following table for infant contact by consultants and registrars in the last **7 days.** (*last Monday at 6am to today (Monday) at 5.59am)*

For 'staff' ONLY record 'registrar' or 'consultant'
For 'exam/procedure' use: ward rounds, sick baby re-evaluation, IV insertion etc.
Record the exam/procedure even if it has been recorded earlier
Do not fill out the table for other staff/procedures e.g. radiology (we do not need details)

Dates (*dd/mm/yyyy*)	Staff (*consultant or registrar*)	Shift (*day, evening, night*)	Exam/Procedure	Duration (*minutes*)

Date of the second day (*Tuesday*) of the 7 day period (*dd/mm/yyyy*): __ / __ / _____

Shift	Nurse code	Number of other infants	Bedspace number	Swab results (*known or unknown*)
AM (0700 – 1500)				+ -
				+ -
				+ -
				+ -
DAY (0700 – 1900)				+ -
				+ -
				+ -
				+ -
PM (1500 – 2300)				+ -
				+ -
				+ -
				+ -
NIGHT (1900 – 0700)				+ -
				+ -
				+ -
				+ -
NIGHT (2300 – 0700)				+ -
				+ -
				+ -
				+ -

Date of the third day (*Wednesday*) of the 7 day period (*dd/mm/yyyy*): __ / __ / _____

Shift	Nurse code	Number of other infants	Bedspace number	Swab results (*known or unknown*)
AM (0700 – 1500)				+ -
				+ -
				+ -
				+ -
DAY (0700 – 1900)				+ -
				+ -
				+ -
				+ -
PM (1500 – 2300)				+ -
				+ -
				+ -
				+ -
NIGHT (1900 – 0700)				+ -
				+ -
				+ -
				+ -
NIGHT (2300 – 0700)				+ -
				+ -
				+ -
				+ -

Date of the fourth day (*Thursday*) of the 7 day period (*dd/mm/yyyy*): __ / __ / _____

Shift	Nurse code	Number of other infants	Bedspace number	Swab results (*known or unknown*)
AM (0700 – 1500)				+ -
				+ -
				+ -
				+ -
DAY (0700 – 1900)				+ -
				+ -
				+ -
				+ -
PM (1500 – 2300)				+ -
				+ -
				+ -
				+ -
NIGHT (1900 – 0700)				+ -
				+ -
				+ -
				+ -
NIGHT (2300 – 0700)				+ -
				+ -
				+ -
				+ -

Date of the fifth day (*Friday*) of the 7 day period (*dd/mm/yyyy*): __ / __ / _____

Shift	Nurse code	Number of other infants	Bedspace number	Swab results (*known or unknown*)
AM (0700 – 1500)				+ -
				+ -
				+ -
				+ -
DAY (0700 – 1900)				+ -
				+ -
				+ -
				+ -
PM (1500 – 2300)				+ -
				+ -
				+ -
				+ -
NIGHT (1900 – 0700)				+ -
				+ -
				+ -
				+ -
NIGHT (2300 – 0700)				+ -
				+ -
				+ -
				+ -

Date of the sixth day (*Saturday*) of the 7 day period (*dd/mm/yyyy*): __ /__ / _____

Shift	Nurse code	Number of other infants	Bedspace number	Swab results (*known or unknown*)
AM (0700 – 1500)				+ -
				+ -
				+ -
				+ -
DAY (0700 – 1900)				+ -
				+ -
				+ -
				+ -
PM (1500 – 2300)				+ -
				+ -
				+ -
				+ -
NIGHT (1900 – 0700)				+ -
				+ -
				+ -
				+ -
NIGHT (2300 – 0700)				+ -
				+ -
				+ -
				+ -

Date of the seventh day (*Sunday*) of the 7 day period (*dd/mm/yyyy*): __ /__ / _____

Shift	Nurse code	Number of other infants	Bedspace number	Swab results (*known or unknown*)
AM (0700 – 1500)				+ -
				+ -
				+ -
				+ -
DAY (0700 – 1900)				+ -
				+ -
				+ -
				+ -
PM (1500 – 2300)				+ -
				+ -
				+ -
				+ -
NIGHT (1900 – 0700)				+ -
				+ -
				+ -
				+ -
NIGHT (2300 – 0700)				+ -
				+ -
				+ -
				+ -

Section 2: Information from mother's recall

This section requires the mother's participation. It requires you to ask the mother for information.

A. Questions to ask the mother.

1. If the infant was fed Human Breast Milk in the last **7 days**, did the mother experience any of the following? (*last Monday at 6am to today (Monday) at 5.59am)*
   (*tick all that apply*):
   ☐ Maternal mastitis ☐ Breast abscess ☐ Nipple cracking ☐ None apply

Comments:_____
_____
_____
_____
_____
_____

Appendix 4: Prospective study weekly CRF guidelines

Guidelines for completing the CRFs.

General guidelines:

- If there is a yes/no option, make sure you select one, do not leave unanswered.
- If there is an 'other' tickbox, make sure you tick 'yes' or 'no'.
- If 'yes' is ticked for 'other' you must specify what the procedure, equipment or medication etc. is that was not provided in the list.
- Pay attention to the time frames (either 24 hours or 7 days). Do not include anything that happened outside that time frame.
- For dates in most of the tables you can write 'ongoing' if the dates are outside the 7 day period. E.g. if the infant has been in the same incubator before and after the 7 day period, write 'ongoing' in both start and stop date columns.

Baseline – Section 1.

A. Demographic information – Infant.

All information for section A will be in the infant's case notes.

- Fill out both date of birth and date of admission, even if they are the same.
- Gestational age is the number of weeks and days the infant was gestating before the birth date. This is not for the current gestation.
- For 'sibling of the neonate' only include siblings from other births. There is a question for multiple births (this pregnancy) in the next section.
- When recording number of siblings only include living siblings.

B. Birth history.

All information for section B will be in the infant's case notes.

- If 'yes' for multiple births, we need the ID for the other infant participants. IDs are printed on the case report forms for the other infants.
- Birth location will be in the infant's notes, but you can ask the mother if it has not been recorded.
- If you tick 'other' for not born in Dunedin Hospital or another health care facility, please specify where the infant was born. For example, at home.

Baseline – Section 2.

Maternal history will be in the infants notes, or the mothers memory (e.g. for 'maternal infection' she will know if she has had a UTI).

Case Report Form – Weekly

Information will be found in the infant's case notes. Ask the nurses assigned to the infant if you are unsure about any of the notes. There is one question (part H), that requires you to ask the mother for information.

A. Infant medical history.

There will be room for additional comments at the end of this section. This can be used if there is not enough room to write on the lines provided.

Comorbidities in the last 7 days.

- Make sure you only tick 'yes' if the onset happened in the last 7 days (last Monday to Sunday). Do not tick 'yes' if it started before last Monday.
- If the infant has not been screened for the comorbidity yet, please tick 'no'.
- Cardiac abnormalities, Pulmonary disease and Gastrointestinal disease: If you tick 'yes', make sure you specify exactly what the abnormality/disease is.
- If you tick 'yes' for other, please specify what comorbidity the infant has.

Infant procedures in the last 7 days.

- Make sure you only tick 'yes' if the onset happened in the last 7 days (last Monday to Sunday). Do NOT tick 'yes' if it started before last Monday.
- Endotracheal (re)intubation: this is if the infant has been re-intubated in the last 7 days. If 'yes', specify the total number of times.
- Blood tests: how many total they have had in the past 7 days.
- Phototherapy: write the total duration in hours of the phototherapy session. If there has been more than one session, add together the duration from both session. Tick if phototherapy included a bili blanket or not (yes/no).
- Syringe pump: tick if the pump was used inside or outside of the incubator, or both.
- For 'other devices': specify what the other procedure/device was and remember you must include if the procedure was inside or outside the incubator for each procedure.
- Remember to include the incubator asset number if you have ticked 'yes' for any procedure requiring a ventilator (specified on the case report form).

B. Medication history.

Antimicrobials, other medication and injectables.

- If the infant received antimicrobials/other medications/injectables in the last 7 days (ticked yes), please fill out the table(s).
- Must include the start date and the stop date or 'ongoing'.
- If the infant begun the antimicrobial treatment before the 7 day period write 'ongoing'. If the infant is still receiving the antimicrobial (it has extended past the 7 days) also write 'ongoing'.
- Injectables do not need to include the route.
- There is room for any extra comments about medications in the past 7 days.

C. Parental/family contact

Contact refers to any physical contact with the infant, inside or outside the incubator.

- Duration is a time frame: short, medium or long (as explained on the case report form). We do not need a specific number of minutes.
- Please specify the type of contact that occurred e.g. skin to skin contact.
- You can put multiple dates in one row if it was the same contact (mum or dad), contact type and duration, otherwise please use different rows.
- To comment on how much contact there was between the parents and infant in the last 24 hours, say if there was a little, medium or a lot of contact. You may need to ask the nurse/s that have looked after the infant over this time. This question is to ensure the completed table reflects how much contact actually occurred.

Infant/multiple.

- Only answer this if the infant is a part of a multiple birth. If they were not then skip question 3.
- Contact can be inside or outside the incubator.
- In the 'other infant' column, write the participant ID for the other infant. This will be printed on the case report form for that infant.

There is room for any extra comments about parental/family contact at the end of this section.

D. Enteral feedings

<u>Last 7 days.</u>

- Infants are usually fed at regular intervals throughout the day. To answer Q1 multiply their feeding in a day by 7. For example, if they're fed every 2 hours they would be fed 84 times in the past 7 days.
- Infants are usually fed at regular intervals so the number of times they are fed a day should be routine over the last 7 days.
- If the routine of the infants feedings changed in the last 7 days (e.g. fed every 3 hours instead of every 2), please record the date that this changed or tick 'irregular changes'.
- Donor human breast milk: this will be less common than mother's breast milk so the question asks for the last 7 days. Include number of times the infant was fed donor human breast milk.

<u>Last 24 hours.</u>

Breast milk

- Number of times the infant was fed breast milk in the last 24 hours.
- Only need to enter the date in the first date column as it will be the same for all of the feeds.
- Do not include donor breast milk in the table.
- 'Number of feeds' column is for the number of feeds of the same type, route and duration.

Formula.

- The number of times the infant was fed formula is for the last 24 hours.
- Only need to enter the date in the first date column as it will be the same for all of the feeds.
- 'Number of feeds' column is for the number of feeds of the same type (ready to feed or mixed by staff) and duration.

E. Environment

This section asks for information from the last 7 days.

Bedspace, incubator, cot, and stethoscope.

- If the infant has been in the same bedspace/incubator/cot/stethoscope for more than the 7 day period, the start date will be 'ongoing' and the stop date will be 'ongoing'.
- If the infant has changed bedspace/incubator/cot/stethoscope in the last 7 days please fill out both available rows on the table so we have a record of both bedspace numbers and when the change occurred. Make sure the dates are recorded.

Weight.

If the infant has been weighed in the last 7 days complete the table.

- This should happen three times a week so there should be three records in the table.
- Write whether the scale is inbuilt or external. Do not need to record the number of the scales.

Equipment.

- If 'yes' is ticked for 'other' make sure the type of equipment is recorded.
- Record if the thermometer was shared or the infants own, and the asset number.

F. Estimated infant contact

Cares.

- Cares over a 24 hour period is routine. If the infant needs cares every 3 hours this will equal 08 cares.
- Family/parental contact: If there has been any parent contact for cares in the last 24 hours record the family members involved. Only record 'mother', 'father' as other family members are not usually involved. Do not record the mother or father in any other way e.g. do not write their first or last names.
- Make sure the contact person and type of cares that contact was used for (e.g. nappy change) is recorded.

Apnoeas requiring stimulation.

- Period we are investigating is 24 hours.
- Can tick more than one intervention type.
- If 'other' is selected, please specify what other equipment was used.

Other nursing procedures.

- Other nursing procedure would include anything not already covered e.g. suppositories and urine collection.
- Specify the procedures and the total number in the last 24 hours.

Other procedures.

- Tick 'yes' or 'no' for the other procedures, do not leave anything blank. If you tick 'other' specify what other procedure (not already included) the infant was involved in.

G. Consultant and registrar contact

This section is for contact of consultants and registrars.

- In the 'staff' column write only 'consultant' or 'registrar'. Do not write their first or last names.
- Record which shift the staff member was working so that their code number can be found.
- Record the type of exam/procedure that the staff member was involved in, even if it has been recorded earlier, the duration of the procedure and the date.
- Can put multiple dates in one column if it is the same staff, shift, exam and duration.

H. Nurse contact

This section is for contact of nurses.

- In the 'nurse' column write the nurses code.
- For 'number of other infants,' record the number of other infants in the nurse's workload.
- Record the other infants in separate rows e.g. if there are two others fill in the first row with '1' and the second row with '2'.
- For 'swab results', record if the S. capitis test result is 'known' or 'unknown.'
- If the test result is known (the infant has a result from the previous week) circle + for a positive result or circle – for a negative result.
- If the test result is unknown (the infant has not had a result from the previous week) do not circle + or -

I. Questions to ask the mother.

- The question regarding mastitis and other conditions of the breast while breast-feeding needs to be answered by the mother.

Appendix 5: Retrospective study baseline CRF

Case Report Form Participant ID: R _ _ _

Case Report Form – Baseline

A. Demographic information

1. Is the infant eligible for the study? ☐ Yes ☐ No

   No: why is the infant not eligible?
   ☐ Not swabbed their first week in the NICU
   ☐ Not in the NICU for a full week (Monday to Monday)
   ☐ Only swabbed once ☐ Positive first swab ☐ Other: _____

   No: did the infant have a positive swab during their stay in the NICU? ☐ Yes ☐ No

   If yes, what was the date of the first positive swab? (*dd/mm/yyyy*): __ /__ /____

2. Were the paper notes available for this infant? ☐ Yes ☐ No

   No: why were they unavailable? ☐ Asbestos in store room ☐ Other: _____

   Not available but eligible: How many weeks of data would we collect if the notes were available: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8+

   Not available but eligible: did the infant have a positive swab during their stay in the NICU? ☐ Yes ☐ No

   If yes, what was the date of the first positive swab? (*dd/mm/yyyy*): __ /__ /____

3. Sex (*must tick one*): ☐ Male ☐ Female

4. Date of birth (*dd/mm/yyyy*): ___ /___ /_____

5. Date of admission (*dd/mm/yyyy*): ___ /___ /_____

6. Date of discharge (*dd/mm/yyyy*): ___ /___ /_____

7. Status at discharge: ☐ Alive ☐ Deceased

8. Reason for discharge: ☐ Ready for home ☐ Transferred

   If transferred, where: _____

9. Was the infant re-admitted to the NICU? ☐ Yes ☐ No

   If yes, date of admission and discharge (*dd/mm/yyyy*):
   ___ /___ /_____ to ___ /___ /_____

   If yes, status at second discharge: ☐ Alive ☐ Deceased

If yes, reason for second discharge: ☐ Ready for home ☐ Transferred

If transferred, where: _____

10. Gestational age at birth (*fill in the number of weeks as well as the additional days*):
____ weeks ____ days

11. Birth weight: _____ grams

12. Infant ethnicity (*tick all that apply*):
☐ NZ European ☐ Māori ☐ Samoan ☐ Cook Island Māori ☐ Tongan
☐ Niuean ☐ Chinese ☐ Indian ☐ Other: _____

13. Does the neonate have siblings from other births? ☐ Yes ☐ No

If 'yes', number of living siblings from other births (*e.g. 01, 02, 03*): ____

B. Birth history

1. Delivery type (*must tick one*): ☐ Caesarean section ☐ Vaginal delivery

Was the delivery an emergency? ☐ Yes ☐ No

If the infant was born by vaginal delivery, were any of the following included?
(*tick all that apply*): ☐ Forceps ☐ Ventouse ☐ None apply

2. Did this pregnancy involve multiple births? ☐ Yes ☐ No

If 'yes', how many births? (*e.g. triplets = 3*): ☐ 2 ☐ 3 ☐ 4 ☐ 5

3. Was the infant born in Dunedin Hospital: ☐ Yes ☐ No

If 'no', where was the infant born? (*the closest town or city*): _____

If 'no' (*select only one*): ☐ Health care facility ☐ Other (*e.g. at home*): _____

If 'yes' to health care facility, what facility? (*e.g. hospital, birthing facility*):
_____

Appendix 6: Retrospective study weekly CRF

Case Report Form Participant ID: _ _ _

Case Report Form – Weekly

Participant ID: R __ __ __

Date of the Monday of the week of interest (*dd/mm/yyyy*): __ /__ /____

Result from the *S. capitis* swab test: ☐ Positive ☐ Negative

Date of the *S. capitis* swab test (*dd/mm/yyyy*): __ /__ /____

A. Infant medical history

1. Comorbidities in the last **7 days** (*last Monday at 6am to today (Monday) at 5.59am)*

Tick either yes (Y) or no (N) for each of the following if the onset is in the last 7 days (do not leave blank).

Y	N		Y	N	
☐	☐	Sepsis workup	☐	☐	Renal impairment
☐	☐	Apnoea of prematurity	☐	☐	Retinopathy of prematurity (ROP)
☐	☐	Feed intolerance (feeds workup)	☐	☐	Sepsis diagnosis
☐	☐	Gastric aspirate (bile present)	☐	☐	Skin injury
☐	☐	Hypoglycemia	☐	☐	Area of inflamed skin: _____
☐	☐	Neonatal encephalopathy	☐	☐	Umbilical flare
☐	☐	Patent ductus arteriosis	☐	☐	Flaky skin
☐	☐	Temperature instability			
☐	☐	Intracran. hemorrhage. *If 'yes' grade*: ☐ 1 ☐ 2 ☐ 3 ☐ 4			
☐	☐	Cardiac abnormalities (*e.g. congenital heart disease*): _____			
☐	☐	Pulmonary disease (*e.g. CLD, HMD/RDS, meconium aspiration*): _____			
☐	☐	Gastrointestinal disease (*e.g. NEC, gastroschisis, omphalocele*): _____			

2. Did the infant have any of the following procedures or devices *in situ* in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)*

Tick either yes (Y) or no (N) for each of the following if the procedure was in the last 7 days (do not leave blank).

Y	N		Y	N	
☐	☐	Endotracheal (re)intubation	☐	☐	Supplemental O_2
☐	☐	Invasive mechanical ventilation	☐	☐	Retinopathy of prematurity screen
☐	☐	Nasal CPAP	☐	☐	Umbilical Artery Catheter (UAC)
☐	☐	Nasal cannula (Optiflow)	☐	☐	Umbilical Vein Catheter (UVC)
☐	☐	Peripheral IV	☐	☐	Peripheral Inserted Central Catheter
☐	☐	Orogastric tube			
☐	☐	Nasogastric tube			
☐	☐	Blood tests. *If 'yes,'* total number: _____			
☐	☐	RBC transfusion. *If 'yes,'* total number: _____			
☐	☐	Phototherapy. *If 'yes,'* duration: _____ days (*total*)			
☐	☐	Syringe pump. *If 'yes,' tick all that apply*			
☐	☐	Other devices (*must include if the device is inside or outside the incubator*): _____			

B. Medication history

1. Was the infant treated with any antimicrobials in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No
   - *If 'yes', fill out the following table.*

IV – intravenous, PO – per oral, PR –per rectal
Start date/ongoing - If the infant started receiving the treatment before the 7 days period begun, write 'ongoing'
Stop date/ongoing - If the infant is still receiving the treatment write 'ongoing'

Antimicrobial	Route (*IV, PO, PR, topical*)	Start date/ongoing (*dd/mm/yyyy*)	Stop date/ongoing (*dd/mm/yyyy*)

2. Other medications, excluding injectables, received in the last **7 days**. (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No
   - *If 'yes', fill out the following table.*

IV – intravenous, PO – per oral, PR –per rectal
Start date/ongoing - If the infant started receiving the treatment before the 7 days period begun, write 'ongoing'
Stop date/ongoing - If the infant is still receiving the treatment write 'ongoing'

Medication	Route (*IV, PO, PR, topical*)	Start date/ongoing (*dd/mm/yyyy*)	Stop date/ongoing (*dd/mm/yyyy*)

3. Other injectables received in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No
   - If 'yes', fill out the following table.

Product example - TPN (Total Parental Nutrition), Lipids, 10% dextrose, insulin.
Start date/ongoing - If the infant started receiving the treatment before the 7 days period begun, write 'ongoing'
Stop date/ongoing - If the infant is still receiving the treatment write 'ongoing'

Product	Start date/ongoing (*dd/mm/yyyy*)	Stop date/ongoing (*dd/mm/yyyy*)

C. Enteral feedings

The last 7 days: (*last Monday at 6am to today (Monday) at 5.59am)*

1. How many times was the infant fed in the last **7 days**? _____

What type of food was the infant fed in the last **7 days**? (*choose all that apply*):
☐ Breast milk ☐ Expressed breast milk ☐ Formula ☐ Donor breast milk
☐ Other: _____

Was fortifier used in the last **7 days**? ☐ Yes ☐ No

What route was used for feeds in the last **7 days**?
☐ Breast feeds ☐ Bottle ☐ NGT ☐ OGT ☐ Syringe ☐ Other: _____

D. Environment

The last 7 days: (*last Monday at 6am to today (Monday) at 5.59am)*

1. Bedspace number

Start date/ongoing - If the infant is in the same bedspace from before the 7 day period began, write 'ongoing'
Stop date/ongoing - If the infant is in the same bedspace (post 7 day period) write 'ongoing'

Bedspace number (*e.g. 01, 02, 03*)	Start date and time/ongoing (*dd/mm/yyyy, 24hr time*)	Stop date and time/ongoing (*dd/mm/yyyy, 24hr time*)

2. Was the infant in an incubator or a cot in the last **7 days**? ☐ Incubator ☐ Cot

If the infant moved to a cot in the last **7 days**, when was the change? (*dd/mm/yyyy*):
___ /___ /_____

3. Was the infant weighed in the last **7 days**? (*last Monday at 6am to today (Monday) at 5.59am)* ☐ Yes ☐ No

Please fill out for every time the infant was weighed (usually three times a week)

Weight (*grams*)	Date (*dd/mm/yyyy*)

E. Apnoeas requiring stimulation

1. Has the infant had any apnoeas requiring stimulation in the last **7 days**?
☐ Yes ☐ No

If 'yes,' number in incubator in the last **24 hours** (*e.g. 01, 02, 03*): _____

Intervention type (*tick all that apply*):
☐ Gentle ☐ Vigorous ☐ Bag and mask ☐ Neopuff (Ambubag)
☐ Funnel/facial O2 ☐ Other: _____

F. Other procedures

1. Other procedures in the last **7 days** (*last Monday at 6am to today (Monday) at 5.59am)*.

Tick either yes (Y) or no (N) for each of the following (do not leave blank).

Y N

☐☐ Abdominal ultrasound
☐☐ Echo cardiogram
☐☐ Eye check
☐☐ Head Ultrasound Scan (HUS)
☐☐ Scans
☐☐ X-ray
☐☐ Other: _____

Appendix 7: Prospective study participant consent form

Risk factors of *Staphylococcus capitis* colonisation, Neonatal Intensive Care Unit, Dunedin Hospital.

Co-ordinating Investigator: Louise Thorn, Masters student

(louise.m.thorn@gmail.co.nz, Ph 0273111874)

Co-investigators: Prof John Crump, Dr James Ussher, Associate Prof Roland Broadbent and Associate Prof Katrina Sharples

CONSENT FORM FOR PARTICIPANTS

Following signature and return to the research team this form will be stored in a secure place for ten years.

Name of infant participant:……

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people of my choice about participating in the study.
3. All my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
4. I know that my child/dependants participation in the project is entirely voluntary, and that I am free to withdraw from the project at any time without disadvantage.
5. I know that as a parent or guardian of the infant participant I consent to data from the weekly *S. capitis* screening being used in the study. I also consent to clinical data and medical information being collected.
6. The mother of the neonate provides consent to their antenatal and demographic data being collected Yes ☐ No ☐
7. I consent to a faecal sample from my child being collected Yes ☐ No ☐
8. I consent to clinical staff being informed of my child's participation in the study

1. I know that all samples and clinical information will be stored in a de-identified form (ie myself or my child will not be able to be identified from them).
2. When the project is completed all the paper records and electronic files which represent the data from the project will be placed in secure storage and kept for at least ten years.
3. I understand that the results of the project may be published and be available in the University of Otago Library, but that but my anonymity will be preserved.
4. I know that there is no remuneration offered for this study, and that no commercial use will be made of the data.
5. I am aware some Iwi disagree with storage of tissue or blood samples citing whakapapa, and advise their people to consult prior to participation in research where this occurs, and that it is acknowledged that individuals have the right to choose to participate.
6. I would like to receive the results of the study at its completion. Yes ☐ No ☐

   If "Yes", please provide your email address: _____

I agree for my child to take part in this project.

....
(Signature of Parent/Guardian) (Date)

....
(Signature of Witness) (Date)

.....
(Name of Witness)

I agree to take part in this project.

....
(Signature of Mother) (Date)

....
(Signature of Witness) (Date)

.....
(Name of Witness)

This project has been approved by the Health and Disability Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the University of Otago Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Appendix 8: Prospective study participant information sheet

Participant Information Sheet

Study title:	**Risk factors of *Staphylococcus capitis* colonization, Neonatal Intensive Care Unit, Dunedin Hospital**	
Coordinating investigator:	**Louise Thorn**, Department of Preventive and Social Medicine, University of Otago Email: louise.m.thorn@gmail.com	Contact phone number: 027 311 1874
Co-investigators:	**Associate Prof Roland Broadbent,** Women's and Children's Health. **Prof John Crump,** Department of Preventive and Social Medicine. **Associate Prof Katrina Sharples,** Department of Mathematics and Statistics. **Doctor James Ussher,** Department of Microbiology and Immunology.	

Introduction

Thank you for your interest in this project. Please read this information sheet carefully. Take time to think about whether or not you want your child to participate in this project and, if you wish, talk with whanau, friends, hapu or iwi. Please be aware that this study is part of a Master's research project. The results will have no immediate benefits to you or your child. However it may contribute to improved health for future premature babies.

If you decide to allow your child to participate we thank you. If you decide not to allow them to participate there will be no disadvantage to you or your child and we thank you for considering our request.

What is the aim of this research project?

The aim of the project is to determine the risk factor for *Staphylococcus capitis* (*S. capitis*) colonisation of neonates (premature babies) in the Dunedin Hospital Neonatal Intensive Care Unit (NICU). *S. capitis* is a type of bacteria that lives on the skin, usually without causing harm (known as colonisation). However, it can cause blood poisoning in susceptible neonates with catheters or other intravascular devices. Low birthweight neonates in NICUs are susceptible to severe disease due to their immature immune systems and their increased requirement for catheters. The Dunedin Hospital NICU has been checking for *S. capitis* on the skin by swabbing the neonate's armpits. This testing has shown that more than a third of admitted neonates get the bacteria on their skin. To control the spread of *S. capitis*, we would like to complete a study to help us understand where the bacteria is coming from and how it gets spread between patients.

Who is funding this project?

Data collection will be hosted by the Dunedin Hospital NICU, who will provide support for participant consent and enrolment, and resources for study materials. Southern Community Laboratories will provide *S. capitis* surveillance testing of the neonates.

Who are we seeking to participate in the project?

We are seeking all neonates admitted to the Dunedin Hospital NICU at under 36 week's gestation. The neonate will be eligible for participation if they are admitted to the NICU for two or more rounds of swabbing (8-14 days minimum post-admission).

If you allow your child to participate, we will also ask the child's mother to participate in the study.

If you participate, what will you be asked to do?

If you agree for your child to participate in this project, we will have access to the results from the routine *S. capitis* test. An armpit swab will be collected from your child as routine procedure in the NICU. If you choose to allow you child to participate, we will have access to the results of the swab. We will continue to collect the data until your child leaves the study or the swab is positive for *S. capitis* bacteria. Faecal samples will also be collected from dirty nappies and tested for *S. capitis* (consent for this will be included on the consent form). Participation also provides consent for clinical data and medical information to be collected by NICU staff. We will inform clinical staff of your child's involvement in the study.

If you, as the child's mother, agrees to participate, clinical staff will ask you some basic demographic questions, questions about your pregnancy and relevant medical history. The clinical staff will also ask for access to your relevant medical records.

Please be aware that you may decide not to take part in the project without any disadvantage to yourself, or your child, of any kind. Participation is entirely voluntary. In particular, your child's clinical care will be not affected by either refusal or agreement to participate.

Is there any risk of discomfort or harm from participation?

There is no risk of discomfort or harm from taking part in this study.

What specimens, data or information will be collected, and how will they be used?

Currently, as part of ongoing surveillance in the NICU, screening swabs are taken from the armpits of infants in the NICU on a weekly basis and sent to Southern Community Laboratories for the identification of *S. capitis* bacteria. This is routine procedure in the Dunedin Hospital NICU. We will collect the data from the testing to determine which infants have *S.capitis* on their skin and which do not. We will also have access to clinical data and medical information for the completion of case report forms. Case report forms collect information on the potential risk factors for the spread of *S. capitis*. The forms will be completed by clinical staff on behalf of your child. One form will be collected as the start of participation and will include the mothers antenatal data and the other will be completed weekly for the duration of your childs participation. We will use this information to inform infection control practices to prevent the risk of *S. capitis* colonisation and associated bloodstream infections.

Pending the availability funding, we will collect faecal specimens. The faecal sample will be used for additional information about the spread of *S.capitis*. If you wish to give consent for the study, however do not consent to the faecal sample, this is presented as an option on the consent form.

Information about age, gender, and ethnicity will be collected. Clinical information that may influence the findings of the study will be collected.

Please be aware that as the parents or guardians of the infant you will have access to the health information that we are collecting. It is routine procedure for the parents to be informed of the results of the screening swab and this will not be affected by participation in this study.

What about anonymity and confidentiality?

The participants will each be given an individual study number. During the study we will be able to identify the participant. However, when data collection is complete we will destroy the link between the participant and their study number, making it impossible to identify the participant from the data. This will also apply to any data collected from the mother.

Results of this project will be published but the data will be completely anonymous. Anonymised data will be submitted to an online public database. At the end of the project all data will be stored for a minimum of 10 years prior to destruction, as required by the University.

If you agree to participate, can you withdraw later?

Yes, you may withdraw your child from the study at any stage during their admission in the Dunedin Hospital NICU. Withdrawal will prevent us from accessing data from any further *S. capitis* testing or case report forms. However, once the data collection is completed it will not be possible to withdraw as it will be impossible to trace the data back to the participant.

Any questions?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Louise Thorn, Master's student
Department of Preventive and Social Medicine, University of Otago
Phone: 027 311 1874
Email: louise.m.thorn@gmail.com

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@hdc.org.nz

For Maori Health Support, or to discuss any concerns or issues regarding this study, please contact Te Ara Hauora (Maori Health Liaison Service) at Dunedin Hospital:

Phone: (03) 4740999 ext 8649

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHICS
Email: hdecs@moh.govt.nz

Appendix 9: Prospective study ethics approval

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

26 May 2017

Miss Louise Thorn
84c Carroll St
Dunedin Central
Dunedin 9016

Dear Miss Thorn

Re:	**Ethics ref:**	**17/NTB/59**
	Study title:	Risk factors of Staphylococcus capitis colonisation, Neonatal Intensive Care Unit, Dunedin Hospital.

I am pleased to advise that this application has been *approved* by the Northern B Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern B Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

— 2 typos on page 1 of the Participant Information Sheet – should be 'weeks' not 'week's' and 'agree' not 'agrees'.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by HDEC before commencing your study.

If you would like an acknowledgement of completion of your non-standard conditions letter you may submit a post approval form amendment. Please clearly identify in the amendment that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see section 128 and 129 of the Standard Operating Procedures at http://ethics.health.govt.nz/home.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

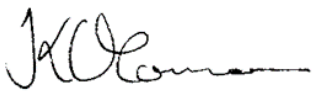
Your next progress report is due by 25 May 2018.

Participant access to ACC

The Northern B Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,



Kate O'Connor
Chairperson
Northern B Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix 10: Prospective study locality approval

Health Research South

COPY

26/06/2017 **Project ID** 01343

Assoc Prof Roland Broadbent
Children's Health, DSM

Dear Roland

SUBJECT: Staphyloccoccus capitis colonisation of neonates at Dunedin Hospital

I am writing on behalf of Health Research South to confirm that the project mentioned above has been granted approval to proceed.

According to our records:

This project is due to commence on: 26/06/2017

It is due to be completed by: 31/12/2018

If you have any questions with regards to this process, please contact me quoting the project ID number shown above.

Yours sincerely

Ruth Sharpe
CLINICAL RESEARCH ADVISOR

C.C. JENNY HUMPHRIES, SOUTHERN DHB
PROF JOHN CRUMP, CENTRE FOR INTERNATIONAL HEALTH, DEPART PREVENTIVE AND SOCIAL MEDICINE, DSM
DR. JAMES USSHER, MICROBIOLOGY, UNIVERSITY OF OTAGO
ASSOC PROF KATRINA SHARPLES, MEDICINE, DSM

Health Research South, PO Box 56, Dunedin 9054
hrs@otago.ac.nz; www.otago.ac.nz/hrs
Ruth Sharpe, Clinical Research Advisor, Ph: 03 470 9032; Ruth.Sharpe@otago.ac.nz

Appendix 11: Prospective study Māori consultation letter

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHAU KI KĀI TAHU

Wednesday, 08 March 2017.

Professor John Crump,
Department of Preventive and Social Medicine,
DUNEDIN.

Tēnā Koe Professor John Crump,

Risk factors for Staphylococcus capitis colonisation in the Dunedin Hospital NICU

The Ngāi Tahu Research Consultation Committee (the committee) met on Tuesday, 07 March 2017 to discuss your research proposition.

By way of introduction, this response from The Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum it states "Ngāi Tahu acknowledges that the consultation process outline in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

As this study involves human participants, the Committee strongly encourage that ethnicity data be collected as part of the research project as a right to express their self-identity. That is the questions on self-identified ethnicity and descent, these questions are contained in the latest census.

The Committee notes and commends that the researchers have undertaken to disseminate the research findings to Māori health organisations regarding this study.

The Ngāi Tahu Research Consultation Committee has membership from:

Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnaka ki Puketeraki
Te Rūnanga o Moeraki

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHAU KI KĀI TAHU

We wish you every success in your research and the committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from Tuesday, 07 March 2017 to 7 September 2018.

Nāhaku noa, nā

Mark Brunton
Kaiwhakahaere Rangahau Māori
Research Manager Māori
Research Division
Te Whare Wānanga o Otāgo
Ph: +64 3 479 8738
Email: mark.brunton@otago.ac.nz
Web: www.otago.ac.nz

Appendix 12: Retrospective study ethics approval

HD16/050

Academic Services
Manager, Academic Committees, Mr Gary Witte

12 September 2016

Dr J Ussher
Department of Microbiology and Immunology
Otago School of Medical Sciences
University of Otago Medical School

Dear Dr Ussher,

I am writing to you concerning your proposal entitled "**Investigation of an outbreak of Staphylococcus capitis in the Dunedin Hospital NICU**", Ethics Committee reference number **HD16/050**.

The above research was submitted and reviewed as a 'Minimal Risk Health Research – Audit and Audit related studies' proposal. The outcome of that consideration was that the Committee was of the view that the study as described is consistent with Rule 11(2) (c) of the Health Information Privacy Code 1994 and was **approved**.

While approving the study please could you confirm whether locality approval is required in order to undertake the work.

The standard conditions of approval for all human research projects reviewed and approved by the Committee are the following:

Conduct the research project strictly in accordance with the research proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee.

Inform the Human Research Ethics Committee immediately of anything which may warrant review of ethics approval of the research project, including: serious or unexpected adverse effects on participants; unforeseen events that might affect continued ethical acceptability of the project; and a written report about these matters must be submitted to the Academic Committees Office by no later than the next working day after recognition of an adverse occurrence/event. Please note that in cases of adverse events an incident report should also be made to the Health and Safety Office:

http://www.otago.ac.nz/healthandsafety/index.html

Advise the Committee in writing as soon as practicable if the research project is discontinued.

Make no change to the project as approved in its entirety by the Committee, including any wording in any document approved as part of the project, without prior written approval of the Committee for any change. If you are applying for an amendment to your approved research, please email your request to the Academic Committees Office:

gary.witte@otago.ac.nz

jo.farrondediaz@otago.ac.nz

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval or an extension of approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor C W Ronson Department of Microbiology and Immunology

Appendix 13: Retrospective study locality approval

Health Research South

9/11/2016 **Project ID** 01282

Dr. Roland Broadbent
Paediatrics

Dear Roland

SUBJECT: Investigation of an outbreak of staphylococcus capitis in the Dunedin Hospital NICU

I am writing on behalf of Health Research South to confirm that the project mentioned above has been granted approval to proceed.

According to our records:

This project is due to commence on: 9/11/2016

It is due to be completed by: 31/12/2017

If you have any questions with regards to this process, please contact me quoting the project ID number shown above.

Yours sincerely

Ruth Sharpe
CLINICAL RESEARCH ADVISOR

C.C.: JENNY HANSON, SOUTHERN DHB
JAMES USSHER, C/O SOUTHERN COMMUNITY LABORATORIES